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Parabiosis for the study of age-related chronic disease

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Summary

Modern medicine wields the power to treat large numbers of diseases and injuries most of us would have died from just a hundred years ago. In view of this tremendous achievement, it can seem as if progress has slowed, and we have been unable to impact the most devastating diseases of our time. Chronic diseases of age such as cardiovascular disease, diabetes, osteoarthritis, or Alzheimer's disease turn out to be of a complexity that may require transformative ideas and paradigms to understand and treat them. Parabiosis, which mimics aspects of the naturally occurring shared blood supply in conjoined twins in humans and certain animals, may just have the power to be such a transformative experimental paradigm. Forgotten and now shunned in many countries, it has contributed to major breakthroughs in tumor biology, endocrinology, and transplantation research in the past century, and a set of new studies in the US and Britain report stunning advances in stem cell biology and tissue regeneration using parabiosis between young and old mice. We review here briefly the history of parabiosis and discuss its utility to study physiological and pathophysiological processes. We argue that parabiosis is a technique that should enjoy wider acceptance and application, and that policies should be revisited especially if one is to study complex age-related, chronic disorders.

Parabiosis – an experimental model inspired by nature

Conjoined twins have fascinated people ever since this naturally occurring physiologic condition gained worldwide publicity through the Siamese brothers Chang and Eng Bunker in the early 19th century. Even though the term "Siamese twins" has been derived from their case, older reports describing conjoined twins date back to the year 1100. The occurrence of this condition is around 1:100000; however, only 26% survive birth [1,2]. The degree of conjunction and the points of attachment vary substantially between different cases and an anatomic terminology has been introduced to classify the types of unions [1,2]. Despite considerable progress in the medical field, the chance of a successful surgical separation of the two individuals still depends on how many vital organs are shared. There are several well-known cases in which a separation of the twins has not been possible or has been declined. For Chang and Eng Bunker, such a surgery was not an option and, therefore, they

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have adapted to living a conjoined life, staying together to an age of 63 years. Conjoined twins develop astonishing coordinative abilities, and for a long time one could only speculate on the physiologic mechanisms underlying this higher form of inter-communication between the two individuals.

In order to investigate the influences of an organism on its conjoined partner, scientists came up with an animal model that essentially copies the natural phenomenon of conjoined twins. The surgical technique to physically connect two living organisms that was later termed “parabiosis” (from the Greek words, para “besides” and bios “life”) was first introduced by the French physiologist Paul Bert in the 1860’s using white albino rats (Fig. 1a). In the beginning, parabiosis surgeries consisted of short skin incisions and a suturing together at the flank of each animal, but the technique has evolved over the years. Nowadays, the skin incisions typically extend along the whole body flank; additionally, in some models the limbs are sutured at the joints and the abdominal walls are joined in order to increase stability and the surface for vascularization. A detailed procedure of the surgery including reversal of the parabiotic pairing has recently been described by Conboy et al. [3]. Following the first experiments using rats, other animal species including axolotls [4]have been included in parabiosis experiments but it turned out that rodents recovered best from the surgery, displaying remarkable resistance against wound infections as opposed to higher mammals. Therefore, the majority of subsequent investigations have been conducted with rats or mice. In addition to connecting adult organisms for parabiosis, embryonic tissue has also been fused in amphibians and fish to study developmental processes (e.g. [5]). Early parabiosis studies using adult animals reported cases of parabiosis intoxication in which one of the two parabionts suddenly died [6,7]. While this intoxication has mainly been due to the lack of genetic uniformity resulting in tissue rejection, a survival rate similar to other invasive surgical procedures (>80%) can now be attained in mouse parabionts with appropriate precautions taken by a skilled operator, and even long-term survival seems unaffected (own observation). So far, most of the parabiosis studies have been conducted in the US and in Japan, whereas only few publications originate from Europe (Fig. 1b).

The early days of parabiosis

In his doctoral thesis “la greffe animale” Bert sutured the skin of two albino rats at their flanks and found that intravenously administered fluids passed from the circulation of one animal into the bloodstream of its adjacent partner. He therefore postulated that surgically connected animals spontaneously develop a single, shared circulatory system through anastomosis (Fig. 2) [8]. For his pioneering work Bert was awarded the Prize of Experimental Physiology of the French Academy of Science in the year 1866. Thereafter, very few studies followed up on his approach until the early 20th century.

In 1908 the German surgeons Sauerbruch and Heyde revived the technique and introduced the term parabiosis for the artificially established symbiosis between two animals [9]. Researchers from a variety of different fields (e.g., endocrinology, metabolism, transplantation, nephrology, radiology, allergy and immunology) started to take advantage of the parabiosis model for their own scientific investigations. A main question at that time was whether transmissible, humoral factors present in one animal have a physiological effect

on its adjacent partner. Rous, who won a Noble Prize in 1966 for his discovery of tumor-inducing viruses, used parabiosis to examine whether the presence of circulating anti-cancer antibodies in tumor-resistant rats would affect tumor susceptibility in attached non-resistant rats. He did not succeed in identifying such protective humoral anti-cancer factors in these experiments [10], but parabiosis was instrumental in his early studies. The most striking results that were obtained using the parabiosis model in this early era have been summarized in an extensive review [11].

More than 1700 articles related to parabiosis have been published (source: http//www.gopubmed.org) since Bert's original dissertation. A publication peak was reached in the years between 1960–1980 (Fig. 1a). In 1969, Coleman grafted mice with the mutation *diabetes* (*db/db*), which are prone to become obese and develop type II diabetes, to inbred wildtype mice [12]. He initially hypothesized that the db/db mouse would lose weight upon exposure to a systemic environment of a non-obese mouse. Surprisingly, he observed that the wildtype mouse significantly decreased food intake while the obese mouse continued to gain body weight. Coleman concluded that there must be a satiety factor involved to which only the wildtype but not the db/db mouse had been able to respond [13]. Almost three decades later, Friedman finally identified this satiety factor and called it leptin [14]. Today, leptin is known as one of the key hormones regulating body weight. Shortly after this remarkable discovery, Friedman and Leibel found that the db gene encodes for the leptin receptor and that mutations in this gene result in a non-functional molecule [15,16]. This finding, which earned Coleman and Friedman the 2010 Lasker award, clearly confirmed Coleman's interpretation of his earlier experiments and underlines the importance of parabiosis models for the identification of new transmissible, humoral factors.

In 1969, another remarkable study using parabiotic pairings was performed by Lewis K. Dahl's group [17]. They grafted wildtype rats to partners with constitutional predisposition for hypertension. As a result they found that renoprival hypertension occurred in both rats at the same frequency. Again this finding pointed towards a humoral factor inducing hypertension in the wildtype animal. Additionally, they described that nephrectomized rats with a predisposition to develop hypertension did not induce higher blood pressure in the wildtype parabiont, suggesting that the factor is produced in the kidney of hypertensinogenic rats. The presence of this factor has subsequently been confirmed in other studies [18] and, in 1993, Lewanczuk et al. identified it as parathyroid hypertensive factor (PHF) [19].

Parabiosis was not only helpful to discover and study individual humoral factors but also to assess the physiological consequences in an organism upon exposure to the systemic environment of its attached partner. Initially, parabiotic surgeries showed highest success rates when using young, sex- and age-matched littermates. Over time the procedure has improved and, in the early 70's, scientists started to graft animals of different ages to each other. This *heterochronic* parabiosis set the basis for the investigation of effects induced through exposure of an aged organism to a youthful systemic environment. In their studies, Ludwig and Elashoff particularly focused on the extension of lifespan in the old *heterochronic* parabiont when attached to a young counterpart. Indeed, in 1972 their results provided the first evidence that the old organism in the *heterochronic* pairing lived longer in response to the young environment compared to the age-matched *isochronic* control animals

[20]. Later, this model proved critical to study the physiology of aging and stem cells in different tissues and organ systems (see below).

Parabiosis for the study of aging and tissue regeneration

In spite of these remarkable findings, based in part on parabiosis, by the end of the last century the procedure had fallen out of favor with the research community with only a handful of papers using the technique (Fig. 1a). It was at that time when Drs. Weissman, Wagers, and Rando “rediscovered” parabiosis at Stanford University for the study of stem cell engraftment and trans-differentiation [21,22] as well as tissue regeneration in the aged organism [23]. Different studies have shown that the regenerative capacity of tissues and organs are dependent on the proliferative activity of progenitor cells derived from tissue-resident stem cells [24–28]. A major hallmark of aging is that the regenerative properties significantly decline in most tissues. This has partially been attributed to impaired stem cell function [29–31]. However, whether these age-related effects were due to cell intrinsic changes or alterations in the microenvironment of stem cells required further investigation. In 2005 Conboy et al. used *heterochronic* parabiosis experiments to address this question. They showed that factors derived from the young systemic environment are able to activate molecular signaling pathways in hepatic or muscle stem cells of the old parabiont leading to increased proliferation and tissue regeneration. These *in vivo* results were furthermore confirmed *ex vivo* by culturing muscle stem cells in medium containing serum from young animals [23]. Their findings clearly suggest that the age-associated impairment of stem cell function is induced to a significant extent by the molecular composition of the surrounding niche rather than by cell intrinsic changes alone.

In 2011 our group published a similar finding suggesting an old systemic environment can be detrimental for stem cell function and negatively regulate adult neurogenesis in brains of young *heterochronic* parabionts. This led to the discovery that factors in old blood are sufficient to decrease synaptic plasticity and impair contextual fear conditioning and spatial memory. Using a systematic proteomic approach (Fig. 2) we were able to identify soluble factors that were significantly increased in blood plasma of old mice and humans. One of these factors was the chemokine CCL11 (eotaxin), known to chemotactically attract eosinophils to tissues. Indeed, application of CCL11 was sufficient to induce impaired adult neurogenesis [32]. Again, these findings provide evidence that the age-related decline in stem cell function can be attributed to changes in the systemic environment. Three more recent publications using *heterochronic* parabiosis further support this conclusion. Ruckh et al. reported that recovery from experimentally induced demyelination in the CNS is enhanced in old mice that were exposed to a young systemic environment [33]. Salpeter and colleagues showed that the decline in pancreatic β-cell proliferation in old mice can be reversed in old parabionts paired with young mice [34]. And most recently, Loffredo et al. demonstrated that age-related loss of normal cardiac function leading to diastolic heart failure is partially due to the lack of certain circulating factors in old mice. They reported that this hypertrophy is reversible upon exposure of an aged animal to a youthful systemic environment through *heterochronic* parabiosis. They identified growth differentiation factor 11 (GDF11), which is significantly reduced in the blood plasma of old mice, as a crucial factor to prevent cardiac hypertrophy.

The promise of parabiosis for regenerative medicine and the study of age-related diseases

The value of parabiosis as an experimental model is most evident for physiological or pathophysiological studies that affect the organism as a whole or that induce changes in the circulatory system. Naturally, such (patho)physiological studies are most relevant to understanding the complexity of higher organisms and disease processes, but they are also the most challenging to conduct and they cannot be replaced by *in vitro* experiments. Indeed, it becomes increasingly evident that many diseases and biological processes, including aging, result in organism-wide, systemic changes contributing to local tissue alterations. Thus, studying an individual organ or cell type in isolation may not lead to a holistic understanding of events. This shift in thinking has been particularly striking with respect to the brain, where decades of neuron-centric research has started to give way to include studies on other brain cell types as critical regulators of cognition and disease, and where a growing number of studies document effects of factors outside the brain including gut microbiota, diet, and other systemic changes on CNS function [35–39].

We think parabiosis is an ideal tool to ask whether alterations occurring in an organism as a consequence of disease, aging, genetic background, infection, diet, exercise etc. might result in circulatory changes altering the status of a healthy, young, uninfected or sedentary organism (Fig. 3). Thus, parabiosis may help assessing the effects of any number of functional states of one organism on a partner organism through a shared circulatory system. This is, of course, only a first step in linking particular factors or cells to a newly discovered transmissible effect. But as the above cited reports show, it has indeed been possible to identify, for example, cells that regenerate an injured brain [33] or proteins that induce satiety [13], regenerate an aging heart [40], or accelerate aspects of brain aging [32]. A generalized approach to reveal such factors or cells using *heterochronic* parabiosis is to analyze systemic changes and correlate them with local alterations in a particular tissue of interest (Fig. 2). Whether the identified candidates are necessary or sufficient to induce pathophysiology may subsequently be assessed by exogenous application or neutralization as well as endogenous overexpression or ablation experiments in suitable animal models.

As many of the major untreatable diseases of our time are chiefly dependent on aging, understanding them will require more insight into the systemic changes and the resulting molecular alterations occurring with age. Animal models can replicate many aspects of chronic diseases including heart disease, stroke, or neurodegeneration, yet we know very little about the contribution of the systemic environment and aging to these conditions. Parabiosis and *heterochronic* parabiosis in particular could help answering some of the fundamental questions in this regard: are circulatory factors or cells in a young organism protecting against age-related disease, and vice versa, are factors or cells in the old organism predisposing or promoting disease in a younger organism? Parabiosis between mutant mice genetically manipulated to develop disease and age-matched or *heterochronic* wildtype littermates or between other genetically engineered mice can help address the importance of systemic factors in the disease process. Variations of this paradigm can help elucidate pathways and mediators in many other conditions (Fig. 3).

Conclusion

Parabiosis has led to remarkable biological and medical discoveries over the last decades and over the past few years in particular. Given its success, it is surprising that this model is not used more extensively. Mice adapt remarkably to the paired living as they gain mobility quickly after surgery and start building nests. When performed with the appropriate refinements and considerations, they do not show overtly abnormal behavior and the survival rate is not affected by the new physiologic state. The highlighted studies underline the promise of the parabiosis model to study aging, stem cells, and tissue regeneration but the model can be employed to address many other aspects of physiology or disease. Considering the remarkable rejuvenating impact young mice have on aged tissues in *heterochronic* pairings, we predict that parabiosis will experience another revival over the next years and will hopefully accelerate our progress towards curing the most devastating diseases of our time.

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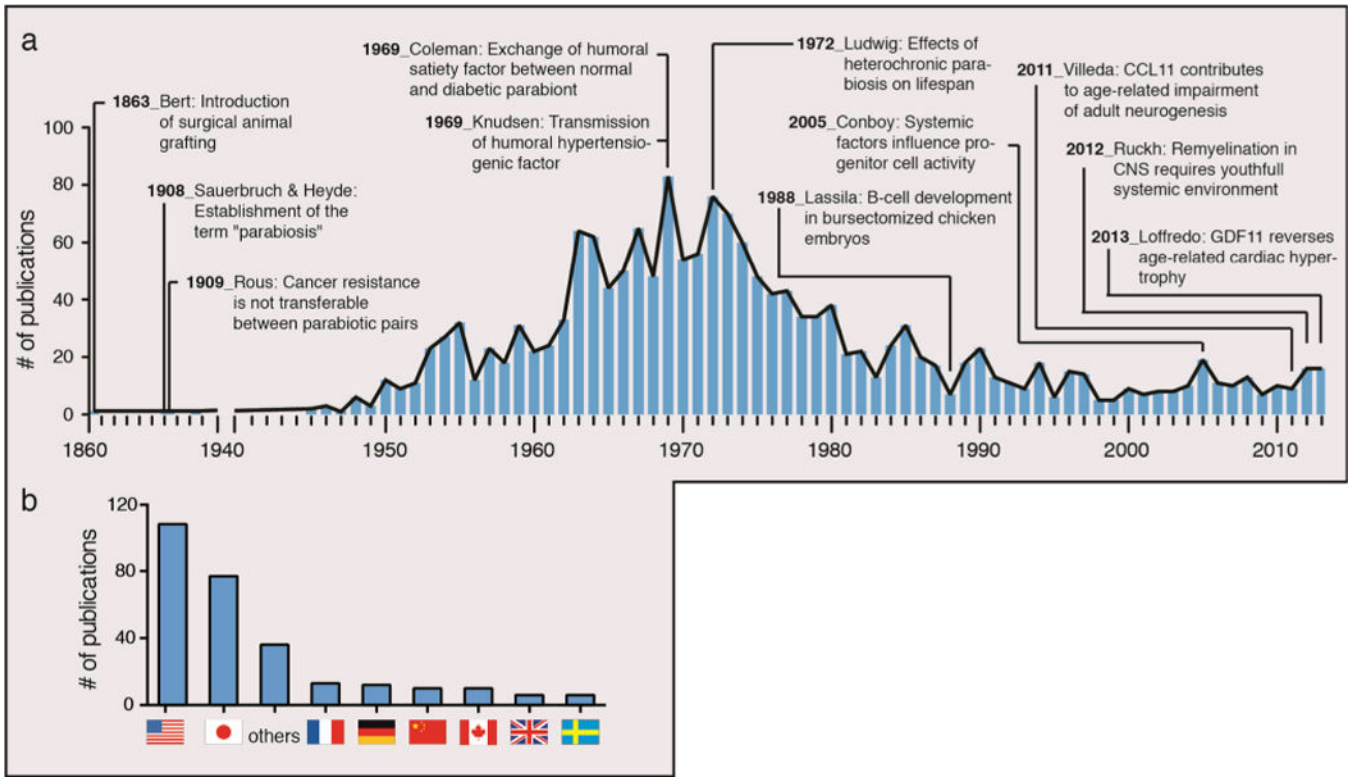

Figure 1. Parabiosis history and modern use

(a) The annual number of publications using parabiosis is shown from 1860–2013. Several studies are highlighted as they provided groundbreaking findings. (b) Publications including parabiosis experiments are listed for different countries. All values have been extracted from http://www.gopubmed.org

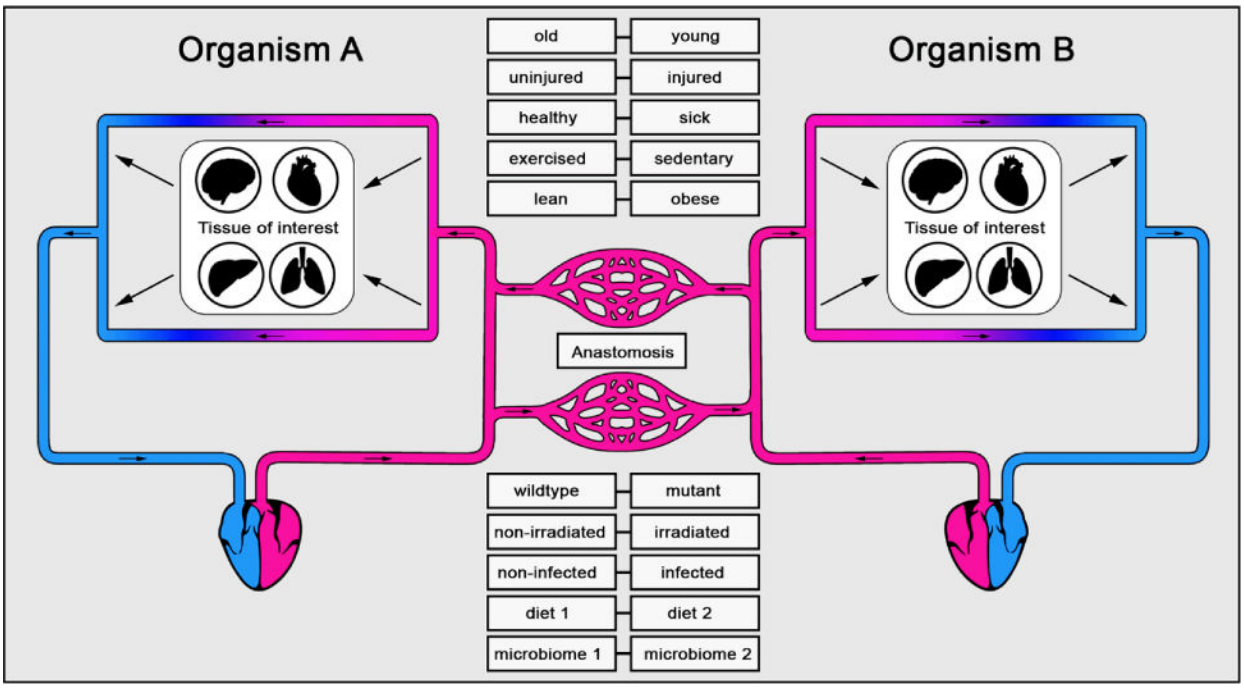

Figure 2. Circulatory system in parabiosis

Organism A and B share a common blood supply, which spontaneously develops through anastomosis post-surgery. Organisms with different physiologic conditions may be used for parabiosis in order to assess the systemic effect of one organism on a particular tissue of interest in its attached partner.

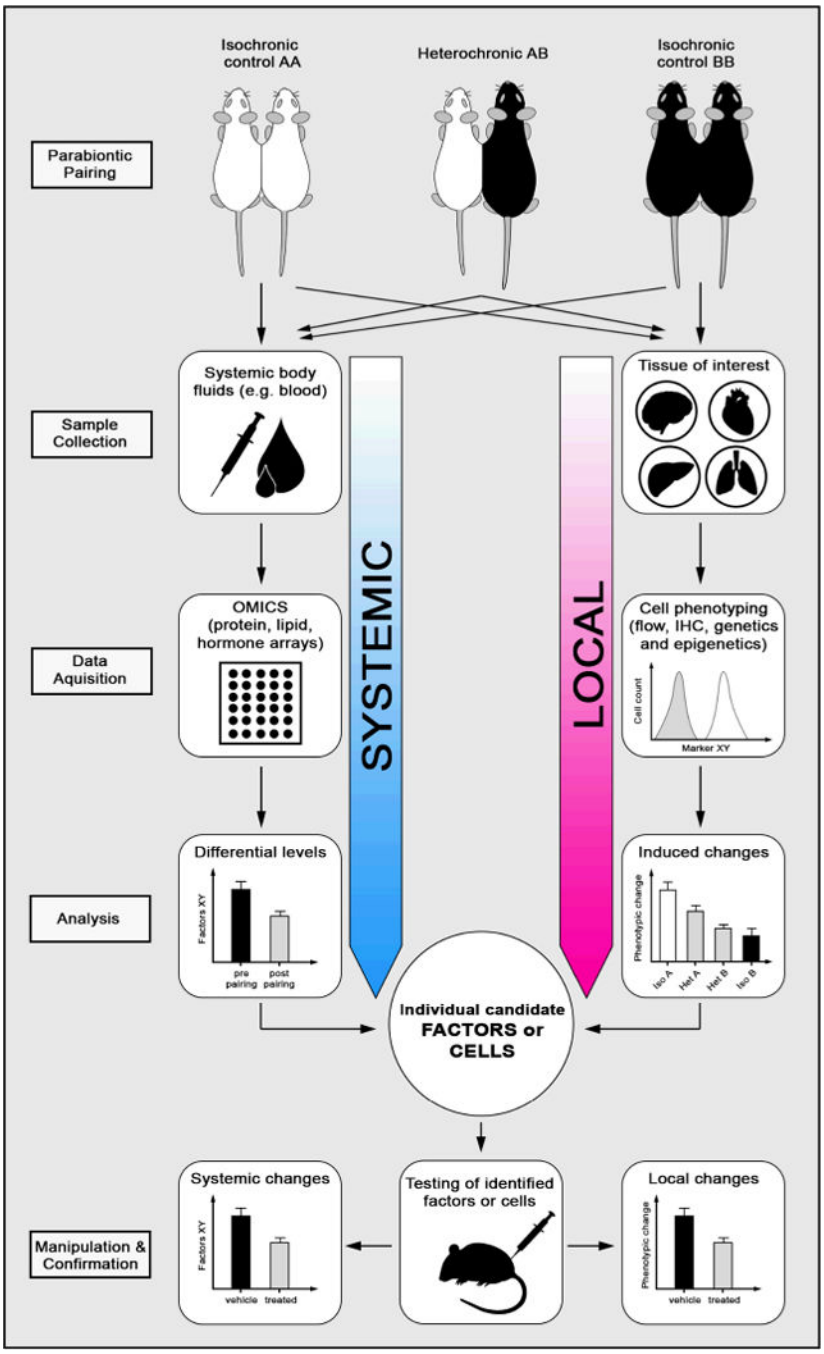

Figure 3. Heterochronic parabiosis

Using *heterochronic* pairings of young (A) and old (B) mice allows assessment of the effect of a young systemic environment on a particular local tissue of interest in the aged partner and vice versa. *Isochronic* pairings (AA or BB) are important controls to exclude surgery-related observations and to determine age-related changes in the systemic environment. Briefly, systemic body fluids such as blood, lymph or CSF are collected, assessed with OMICS tools such as protein, lipid or hormone arrays and analyzed for differential levels of soluble factors pre- and post-parabiosis. A particular tissue of interest is isolated,

phenotypically characterized by flow cytometry, immunohistochemistry or epi-/genetic measures and analyzed for parabiosis-induced phenotypic changes. The integration of these data leads to the identification of individual candidate factors or cells that can subsequently be tested in a suitable mouse model.