NORN GROUP

Hetrochronic Parabiosis

Presented by Madison Ueland on 4/03/2022

Discussion Summary

Paper(s): Primary paper: Conboy, I., Conboy, M., Wagers, A. *et al.* Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 433, 760–764 (2005). https://doi.org/10.1038/nature03260; Historical perspective: Conboy, M. J., Conboy, I. M., & Rando, T. A. (2013). Heterochronic parabiosis: historical perspective and methodological considerations for studies of aging and longevity. *Aging cell*, *12*(3), 525–530. https://doi.org/10.1111/acel.12065

Roadmap

Timeline of parabiosis and aging

Conboy et al., 2005: Rejuvenation of aged progenitor cells by exposure to a young systemic environment

Theories: Dilution or infusion?

Putative factors
- GDF11 debate

Related experiments
- Heterochronic blood exchange
- Neutral blood exchange

Evidence in humans
- Alkahest case study

TIMELINE: Parabiosis vs aging

1863 First parabiosis experiment (Bert, “De la greffe animale”)

1957 Heterochronic parabiosis (HPB) by McCay

1972 HPB extends lifespans of old mice

Early 2000s Parabiosis brought back into fray at Stanford

- **2001** Weissman lab first studied fate and migration of hematopoietic stem cells
- **2005** Rando lab applied to aging: HPB improves regenerative capacity of muscle and liver
- **2011** Wyss-Coray lab extends to cognitive function

2014 Weekly injections of young plasma fail to extend lifespan in mice

2016 Heterochronic blood exchange in mice replicates HPB results

2020 Neutral blood exchange rejuvenates muscle, liver, brain

1993 Cynthia Kenyon daf-2 mutants

1998-2008 Sirtuins / resveratrol controversy

2009 Late life rapamycin extends lifespan of HET-3 mice (NIA ITP)

2011 Removing senescent cells delays age-related diseases

2013 Hallmarks of aging published, Horvath clock

2016 Partial reprogramming extends lifespan of progeroid mice

Ludwig & Elashoff, 1972: Mortality in Syngeneic Rat Parabionts of Different Chronological Age

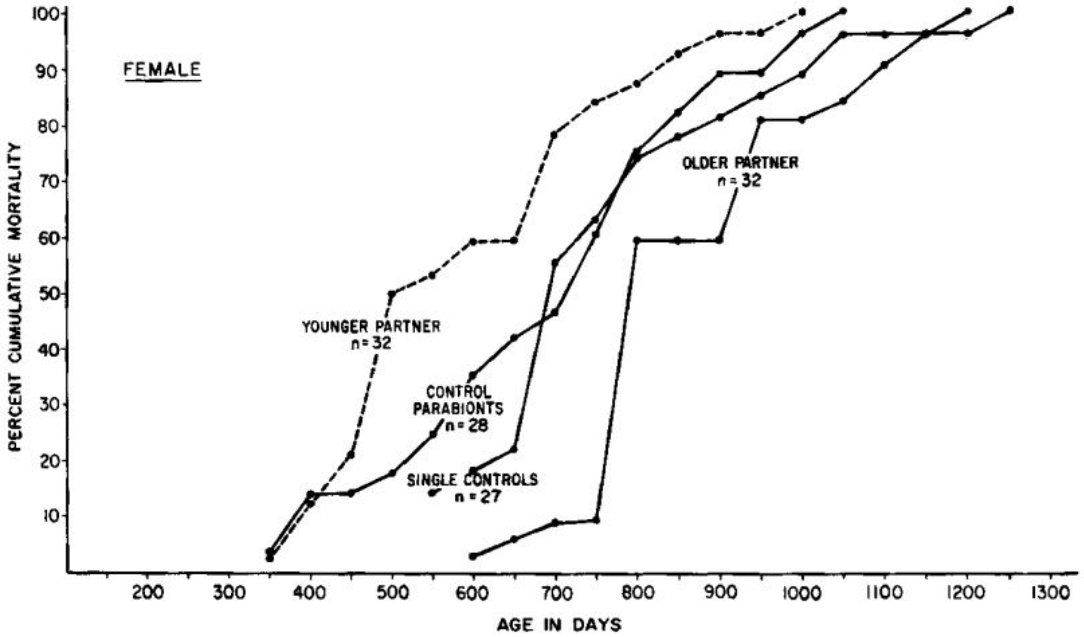


FIGURE 2. Percent cumulative mortality of female single controls, homochronic parabionts, and heterochronic parabionts.

Conboy et al., 2005: Rejuvenation of aged progenitor cells by exposure to a young systemic environment

Known that aging produces decline in regenerative potential due to reduced responsiveness of tissue-specific stem and progenitor cells, and some signalling pathways known to be essential for activation of progenitor cells (e.g. Notch signalling in muscle). Grafting old muscle into young animal more successful than grafting young muscle into old animal.

To what extent is decline in regenerative potential due to stem cell environment, rather than changes in stem cells themselves? In particular, how important is systemic (rather than local) environment, and do blood-borne factors modulate key molecular pathways known to control regenerative properties of progenitor cells?

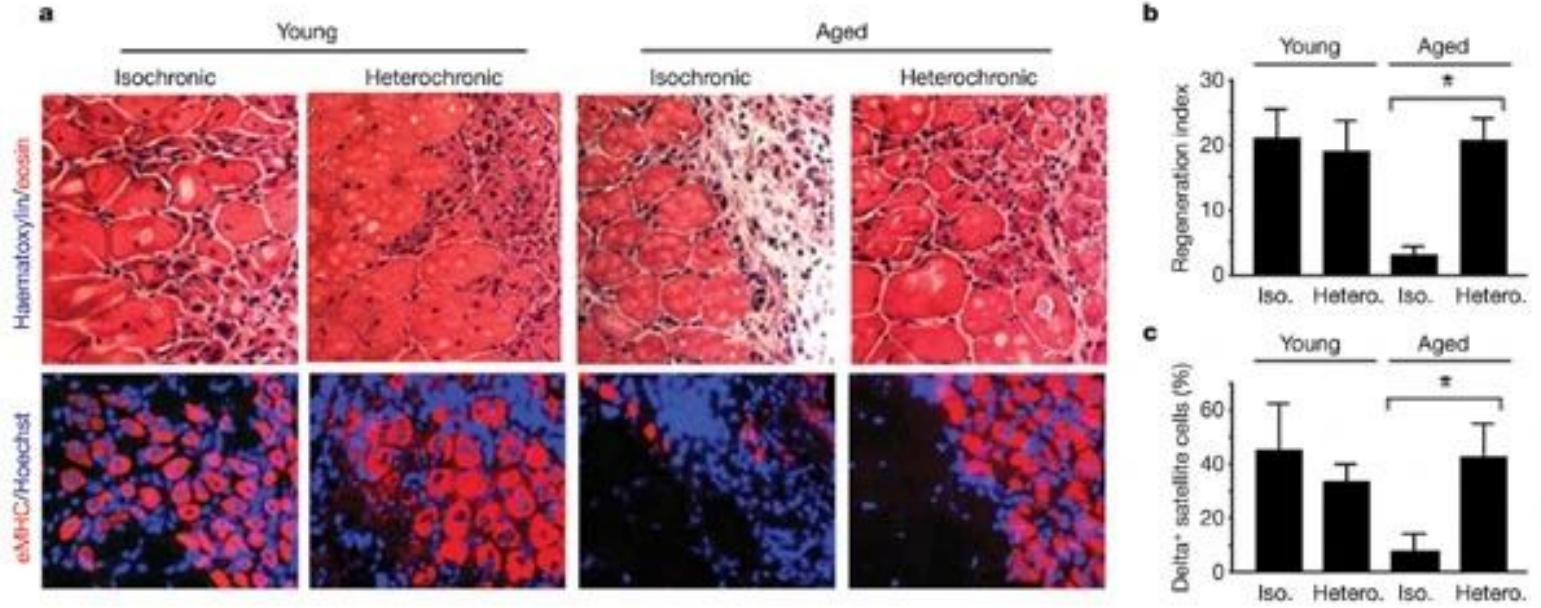
Results:

- Systemic environment matters. Aged muscle and liver stem cells rejuvenate when exposed to younger blood, young stem cells deteriorate when exposed to older blood.
- Tissue regeneration due to amelioration of old stem cells, not migration of younger stem cells.

Conboy et al., 2005: Muscle regeneration

Tested efficacy of muscle regeneration in young (2-3 months) and aged (19-26 months) mice in heterochronic and isochronic pairings

- Hindlimb muscles of each mouse injured after 5 weeks of parabiosis
- Young partners transgenic for GFP so that blood chimaerism could be confirmed, and cells from each animal could be distinguished



+ Less than 0.1% of regenerated myotubes (nascent muscle cells) in aged muscle were GFP-positive, so enhanced regeneration of old muscle due to activation of resident, aged progenitor cells, not engraftment of young circulating progenitor cells
+ Marked upregulation of Notch ligand Delta in satellite cells (skeletal muscle stem cell) of aged heterochronic parabionts, comparable to young (isochronic + heterochronic) parabionts and young mice not subject to parabiotic pairings. Slight inhibition of Delta upregulation in satellite cells in young heterochronic parabionts vs young isochronic parabionts.

“These results indicate that the impaired regenerative potential of aged satellite cells can be improved by a modification of the systemic environment, by means of an **increase of positive factors in young mouse serum, a decrease or dilution of inhibitory factors present in old mouse serum, or both**.”

In addition to muscle regeneration, Conboy et al. examined hepatocyte proliferation, finding parabiosis to young partner significantly increased proliferation in aged mice

Two theories: Dilution or infusion?

Dilution of pro-aging factors vs. Addition of pro-youth factors

+ Not necessarily mutually exclusive
+ Young organs may also play role

How to resolve? Several experiments: Investigate specific factors, exchange blood without sharing organs, dilute old blood without young blood

Putative Factors

Circulating factor	Plasma levels in aged mice	Class	Functions	References
Apelin	Decline	Hormone (Rejuvenating factor)	• Extends the murine healthspan and promotes mitochondriogenesis and protein synthesis	Vinel et al., *Nature Medicine* (2018)
			• Reverses age-associated muscle wasting and delays stress-induced cellular senescence	Rai et al., *Cell Reports* (2017)
β2-microglobulin	Increase	component of MHC class I molecules (Pro-aging factor)	• Impairs hippocampus-dependent cognitive function and neurogenesis	Smith et al., *Nature Medicine* (2016)
Cadherin13	Decline	cell adhesion protein (Rejuvenating factor)	• Inhibits osteoclast differentiation and ameliorates age-associated bone loss	Yang et al. *Aging* (2020)
CCL11	Increase	Cytokine (Pro-aging factor)	• Reduced synaptic plasticity, and impairment of contextual fear conditioning and spatial learning and memory.	Villeda et al., *Nature* (2011)
GDF11	Decline	Cytokine (Rejuvenating factor)	• Reverses age-related cardiac hypertrophy by suppressing phosphorylation Forkhead transcription factor	Loffredo et al., *Cell* (2013)
	Not verified		• Restores the functionality of the vasculature of the neurogenic niches	Katsimpardi et. al., *Science* (2014)
			• Improves muscle physiology and physical function by improving satellite cell competency	Sinha et al., *Science* (2014)
			• Shows calorie restriction–like phenotype	Katsimpardi et al., *Aging Cell* (2020)
			• Increases bone mass by stimulating the BMP signaling pathway, thereby promoting osteogenic differentiation	Suh et al., *PNAS* (2020)
	Increase	(Pro-aging factor)	• Impairs muscle regeneration	Egerman et al. *Cell Metab* (2015)
			• Impairs liver regeneration	Liu et al. Faseb J (2018)
	Not verified		• Induces skeletal muscle atrophy	Hammers et al. *EMBO Mol Med* (2017)
			• Decrease bone mass	Liu et al. Nat Commun (2016)
eNAMPT	Decline	enzyme	• Improves physical activity and extends mouse lifespan by promoting NAD^+ biosynthesis	Mitsukuni et al. *Cell Metab*. (2019)
Oxytocin	Decline	hormone	• Prevent skeletal muscle aging by promoting proliferation of myogenic progenitor cells	Elabd et al. *Nat. Comm*. (2014)
SPARCL1	Decline	matricellular extracellular matrix protein	• Elevated spontaneous synaptic responses, synapse density, and dendritic branching; enhancement of evoked neurotransmission	Kathlyn et al. *PNAS* (2019)
TIMP2	Decline	Protease inhibitor	• Increases synaptic plasticity and hippocampus-dependent cognition	Castellano et al. *Nature* (2017)
THBS4	Decline	matricellular extracellular matrix protein	• Increased spontaneous synaptic responses, synapse density, and dendritic branching; enhancement of evoked neurotransmission	Kathlyn et al. *PNAS* (2019)

Growth Differentiation Factor 11

See Table in Journal Club notes for details

2013, 2014 papers: increasing circulating levels of GDF11 improves heart, muscle and cognitive function in old mice

But in 2015, the story became substantially less clear:

- The reagents used to detect GDF11 were non-specific, may have measured myostatin
- Early mouse studies had shown that GDF11 declined with age, but this was found not to be true
- GDF11 doesn't extend lifespan in progeroid mice

And just as GDF11 data became shaky, evidence supporting the dilution hypothesis emerged with 2016 paper…

Summary of possible interventions

Intervention	Dilution of Pro-Aging Factors	Addition of Pro-Youth Factors	Young Liver and Kidney Function
Heterochronic Parabiosis	YES	YES	YES
Heterochronic Blood Exchange	YES	YES	NO
Neutral Blood Exchange, Plasmapheresis	YES	NO	NO
Injecting Pro-Youth Factor	negligible	YES (but singular factor)	NO
Blocking Pro-Aging Factor	YES (but singular factor)	NO	NO

Heterochronic Blood Exchange

- In HPB, many factors are not well known or controlled:
  - Precise timing: takes 1-2 weeks for vascular connections to develop (Conboy et al., 2005)
  - Amount of blood exchanged
  - In addition to blood, organs are shared, enabling filtering of old blood through young liver, kidney, etc
- HBE: Exchanging blood between mice without surgically joining them
- **2016 study**: Exchanged blood between young and old mice until each had half its blood from the other, then repeated 2005 experiments.
  - Effects on multiple tissues obtained quickly, ~24 hours after exchange
  - “Inhibitory effects of old blood are more pronounced than the benefits of young” (in some cases)
    - Muscle: while regeneration after injury improved in old mice and did not significantly decline in young mice exposed to old blood, performance on four-limb hanging test was diminished for young mice exposed to old blood but not old mice exposed to young blood
    - Brain: hippocampal neurogenesis severely decreased in young mice but no significant positive effect in old mice exchanged with young blood
    - Liver: similar enhancement of old haptogensis and decline of young haptogenesis
- Interpreted by some to validate the dilution theory
- Perhaps explains why weekly injections of young plasma starting at middle age failed to extend lifespan in mice (2014): insufficient dilution?

Neutral Blood Exchange

- To test dilution hypothesis, NBE replaces old plasma with artificial plasma made of a saline and albumin solution, removing the confounding young factors present in HBE
- Less controversial, cheaper, safer than HBE
- 2020 Conboy papers support dilution hypothesis
  - How does NBE compare to HBE? Meets or exceeds rejuvenative effects in muscle, liver, brain
    - Effects of NBE on neurogenesis exceeded those of heterochronic parabiosis experiments (by ~8 times)
  - Is albumin responsible for the improvement? Probably not, doesn't seem to change with more albumin.
  - Many modulated proteins were upregulated despite dilution - illustrates inhibitory effects of old factors (see interesting simplified model in Figure 6)
  - If strong pro-youth factors were responsible for effects of parabiosis, then NBE in young animals would dilute them and impair young animals' regenerative abilities… but no effects seen on muscle repair or liver health.

In Humans?

Companies include:

Alkahest (Wyss-Coray, advised by Rando)
- Plasma injections (GRF6019, GRF6021) formed by pooling blood from young donors and sorting to remove unwanted immune molecules - Phase 2 for Alzheimer's, Parkinsons
- Blockers of pro-aging factors
  - CCR3 inhibitor (AKST4290) blocking CCL11, Phase 2 for age-related macular degeneration and Parkinson's
  - AKST1210: device removing B2M from blood, Phase 2 for end stage renal disease

Elevian (Wagers): GDF11 for stroke

NuGenic Research
- Elixir, another plasma derived product, used in 2020 Horvath preprint to improve epigenetic profile in mice (see also: 2021 Horvath preprint showing HBP improves epigenetic and transcriptomic profiles in mice)

+ There has been some interest in treating Alzheimer's with **Therapeutic Plasma Exchange (TPE)**, which is used to treat several autoimmune diseases, but clinical data is limited e.g. PLASMA study (n=9): too underpowered to draw conclusions.

Technical Challenges

- Operative and perioperative mortality: 10% with anesthesia and postoperative monitoring
- ‘Parabiotic disease’: 20-30% (even in highly inbred strains)
- Takes 1-2 weeks for vascular connections to develop before effects observed
- Both parabionts aging, so age of the ‘effector’ parabiont not controlled