Molecular, cellular and tissular mechanisms of bone catch-up growth

Summary: The control of organ size during development and repair is one of the last frontiers of biology, as we lack basic knowledge on how intra- and inter-organ growth is regulated. The underlying question is common to most biological disciplines: how do cells process intrinsic and extrinsic cues such that their combined behaviours lead to collective outcomes? To address this question, our lab studies the catch-up growth phenomenon (recovery of a normal growth trajectory after a transient insult), using the developing long bones as a model. In this project we will study the compensatory response triggered by local insults in the growth plate (GP), the structure that drives growth of the long bones. Our latest study (*PLoS Biol*, in press; bioRxiv[1]) shows that the response is in part cell-nonautonomous, i.e. the cells (chondrocytes) that were spared hyperproliferate to compensate for the lack of contribution of the affected neighbours. We will explore two **non-exclusive** mechanisms that could underlie this compensatory proliferation (Fig. 1). In the first possibility, a short-range alarm signal (either biochemical or mediated by cell-cell or cell-matrix contacts) is produced by the insulted cells and triggers proliferation of the surrounding neighbours. The type of signal could be different depending on the type of insult. In the second scenario, compensatory proliferation is an all-or-nothing community response, due either to a self-propagated signalling wave, or to interaction with surrounding tissues. Specifically, we will trigger different types of insults in the murine GP, with high spatiotemporal resolution, in order to:

Aim1. Identify and validate the short-range interactions that modulate compensatory proliferation in the growth plate and their dependence on the insult.

Aim2. Dissect the community mechanisms that operate across the growth plate to achieve a coordinated compensatory response to insult, including interactions with the surrounding tissues.

Experimental models and approach: The lab previously developed a genetic strategy (*ePit-Col-p21*) to impair left-limb growth in mice, with the right limb as internal control, by expressing the cell cycle suppressor p21 in >50% of the left chondrocytes. Strikingly, left-right symmetry was maintained, revealing the deployment of compensatory mechanisms. A stress response was activated in the left GP, and associated with hyper-proliferation of spared chondrocytes. We hypothesise that compensatory proliferation involves bottom-up mechanisms in the GP (i.e. cell-cell communication leading to a collective outcome), likely operating within the limits imposed by a top-down mechanism that restricts absolute growth The fact that *ePit-Col-p21* embryos do not exhibit L-R asymmetry at any stage analysed[1] suggests that the system is not reacting to a net change in size (feedback control), but to a more immediate effect of the insult (feedforward control). This control could either operate by proximity, via short-range alarm signals or cell-cell interactions that very likely depend on the type of insult, or in community, via a self-propagated chemical wave, or recruitment of progenitors from an external tissue. Specifically, we will:

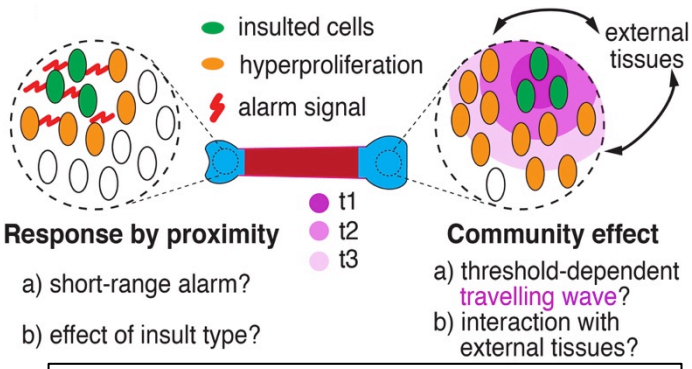

Fig. 1. Proximity and community models for compensatory proliferation in the growth plate (blue). t1-3: time points of the travelling wave.

Aim1.a) Identify and validate the signals and responses that modulate compensatory proliferation, through transcriptomics and proteomics approaches.

Aim1.b) Determine the impact of the injury type on the kinetics of compensatory proliferation using models of unilateral mosaic cell death instead of cell arrest in the GP.

Aim 2.a) Decipher the GP-level cell-cell interactions that underlie compensatory proliferation. Using optogenetics and ex vivo bone culture and imaging, we will trigger p21 in different GP regions of variable positions and diameters, and follow the proliferative response of neighbour cells. The data will be fit into a computational model in collaboration with Prof. Nguyen (Monash U.).

Aim 2.b) Assess the role of extrinsic cells in long-bone development and catch-up growth, using sophisticated lineage tracing experiments combined with inducible injury models.

Necessary background: bioinformatics, imaging, computational modelling and tissue culture.

1. Rosello-Diez, A, Madisen, L, Bastide, S, Zeng, H & Joyner, AL. *bioRxiv*, (2017).