Aging, cell identity, and ubiquitin

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How differentiated cells maintain their identity is a fundamental question in biology. Loss of identity is a hallmark of aged cells and tissues, and is associated with age-related diseases such as neurodegeneration, metabolic disorders and cancer. Our lab is using *Drosophila* as a model system to study the regulation of cell identity during aging focusing on ubiquitin-related genes.

Plasticity of differentiated cells: Long ago, Canard Waddington compared the process of terminal differentiation to that of a ball-rolling downhill into specific valleys of irreversible cell fates [1]. However, over time, the rigid view of "terminally differentiated" cells, was replaced with a more plastic one. For example, nuclear transfer experiments, and forced expression of the myogenic transcription factor MyoD were among the initial experiments demonstrating the fragility of a cell's state [2–5]. More recently, experiments on reprogramming differentiated cells to induce pluripotent stem cells (iPS) established that the expression of four transcription factors (OKSM: Oct3/4, Klf-4, Sox-2, c-Myc) is sufficient for reverting a differentiated cell into a pluripotent state [6]. Yet, reprogramming is an incomplete and inefficient process which indicates that intrinsic barriers exist to maintain identity and prevent re-programming and de-differentiation [7]. As long predicted [8 ,9], in order for differentiated cells to maintain their identity, both passive and active chromatin-related mechanisms are required. Thus, above "the Waddington valleys" is a yet to be identified "safety-net" that supervises the differentiated identity and prevents the differentiated cells from wandering outside their destination.

Aging and Drosophila. Coping with aging-related disease is one of our society's greatest challenges. *Drosophila* is emerging as a powerful model organism for the study of aging. It is a fast and track table system; aged flies are only 3-4 weeks old, and the molecular pathways involved in *Drosophila* aging are highly conserved in humans. Moreover, *Drosophila* offers a powerful genetic toolbox and is excellent for imaging. Thus, making it a wonderful system for unveiling basic principles and regulators involved in cellular, tissue, and organismal aging.

The transcription factor Hey and nuclear lamins are supervisors of enterocytes identity. Using the gut of adult *Drosophila* as a model system, we delineated the core principles and key supervisors that maintain the differentiated identity of fully differentiated midgut enterocytes (ECs). We found that the transcription factor Hey together with nuclear lamins supervise the identity of ECs in the adult Drosophila midgut (10) .

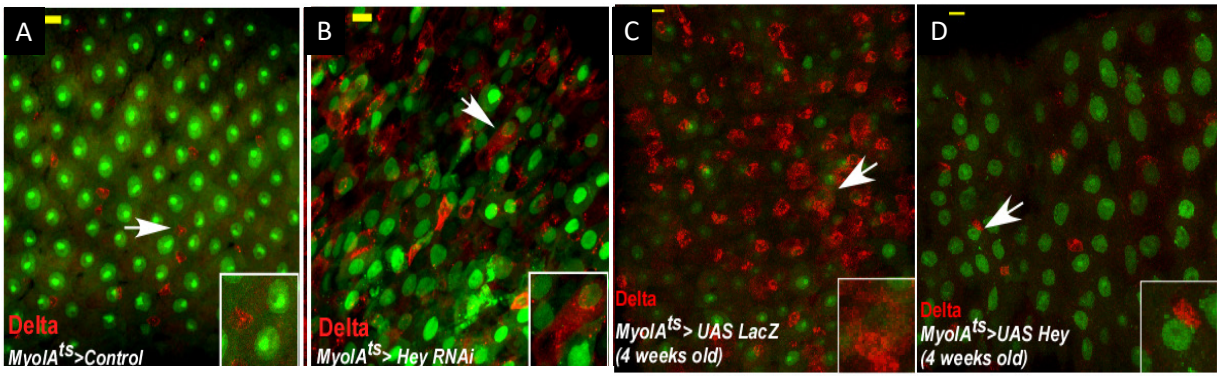

Figure 1: *Hey supervises EC identity and its expression suppresses aging phenotypes.* Confocal images of the adult drosophila midgut. (A) Young (4 days old) midguts. Differentiated enterocytes are marked in Green (MyoIA>GFP). Delta (Red) marks stem cells. In control tissue (A), Delta is expressed only on the surface of stem cells (arrow). (B, C) upon acute targeting of Hey (B) or in aged midguts (C), EC lose the expression of MyoIA>GFP and ectopically express Delta (red) (see arrows). (D) Expression of Hey in ECs of aged flies prevents these changes; restores GFP expression and prevents the ectopic expression of Delta (adopted from 10).

Using lineage tracing of post-mitotic cells (see below), we observed that loss of Hey, or Lamin C, in ECs resulted in inability to maintain EC differentiated identity. These ECs loss the unique nuclear organization of ECs, and the midgut exhibit pathological reprograming of progenitors. Orchestrating the unique nuclear organization of ECs, Hey and Lamins, co-regulate and maintain enhancers' activity, shifting from a stem-cell organization into a differentiated one. Moreover, maintaining this configuration of nuclear lamins is key for cell identity.
During *Drosophila* aging Hey levels declines and EC identity is lost. Similarly, genetic ablation of Hey or ectopic-expression of stem-cell-related Lamin in ECs of young adults overrode EC identity programs, reduced epithelial integrity and organismal survival. Thus, a single transcription factor concomitantly supervises chromatin and nuclear organization, safeguarding cell identity (Figure 2; 10).

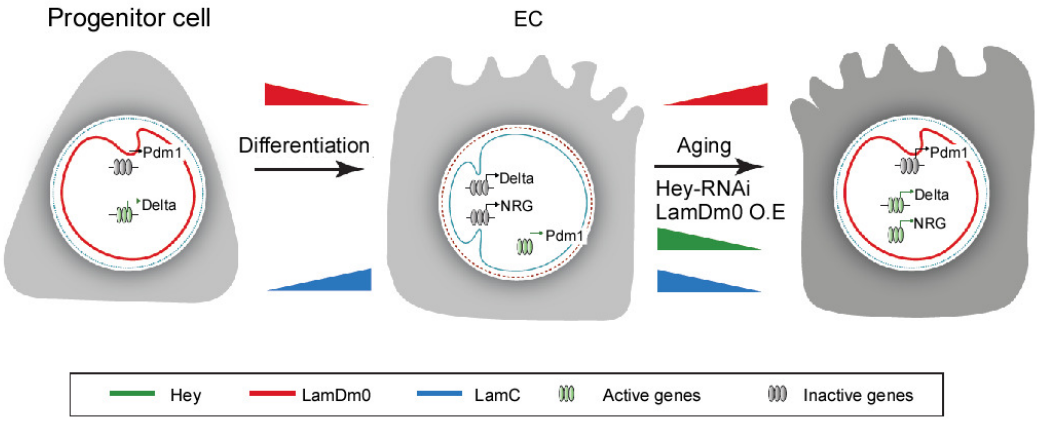

Figure 2: Model for supervising cell identity by Hey and nuclear lamins: Hey regulates EC identity in part by establishing and sustaining a transcriptional switch in the expression of nuclear lamins. In enterocytes stem cells, the dominant lamin is LamDm0, which prevents the expression of differentiated genes. During differentiation and in differentiated ECs, Hey represses the expression of LamDm0, enabling the expression of genes required for EC physiology and function. In addition, Hey promotes the expression of differentiated enterocytes (ECs) gene signatures, including Pdm1 and LamC, the latter inhibiting the expression of stem cell- and non-gut-related genes in ECs. Hey loss during aging or upon its acute genetic ablation in young midguts, results in ectopic expression of LamDm0 and subsequently silencing of EC programs including critical EC TFs (e.g. Pdm1 and Odd-skipped). Concomitantly, Hey loss in ECs causes a decline in LamC and, as a consequence, ectopic expression of stem cell- and non-gut-related genes that are normally repressed by LamC in ECs (Adopted form 10).

Monitoring cell identity using G-TRACE: To determine the fate of individually targeted ECs and to assess the cellular composition of the gut upon Hey loss in ECs, we used "G-TRACE", a method for lineage tracing of non-dividing cells (Figure 3, 11). In brief, a UAS-RFP marker is expressed via the EC-specific promoter/driver MyoAI-Gal4, which is active only in fully differentiated ECs (red). The same MyoIA-Gal4 also drives the expression of a UAS-flipase that induces a recombination event that activates permanent GFP expression, which serves as a "history marker". This GFP "history marker" is expressed in fully differentiated ECs and their progeny regardless of the cell's current differentiation state. All ECs in control guts were both RFP and GFP positive (RFP+, GFP+) and appeared orange (Fig. 3B). In contrast, upon Hey targeting in ECs, we observed diverse fluorescence populations of polyploid cells (PPCs; Figs. 3C')

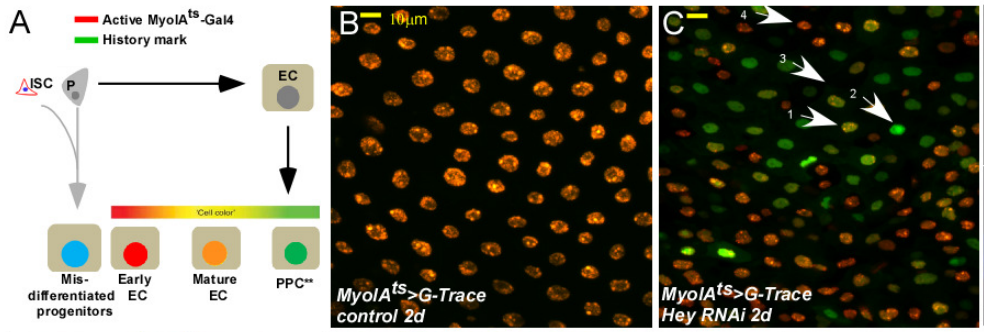

Figure 3: *G-TRACE analysis of Hey targeted ECs*. (A) Illustration of G-TRACE lineage tracing (adopted from Evans et al., 2009). (B-C) Confocal images of G-TRACE analyses of control (B) and Hey-targeted ECs (C). (B) All ECs in control guts co-express MyoIA-Gal4>UAS-RFP, and the history marker Ub::GFP, and are denoted in orange [GFP (+) RFP (+)]. (C) A heterogeneous population of polyploid cells is observed in midguts in which Hey is targeted in ECs for 48h using the indicated Hey-RNAi transgenic lines including fully differentiated $ECs^{GFP(+)RFP(+)}$ (white arrow #1); and EC that are no longer fully differentiated, $EC^{GFP(+)RFP(-)}$; (white arrow #2 adopted from 10).

Identification of ubiquitin-related identity supervisors:

The regulation of ECs identity must involve communicating to the nucleus the physiological changes in the guts' environment by genes regulating signaling and post-transcriptional modifications (PTM). One type of modifications is the covalent attachment of ubiquitin or ubiquitin-like (Ub/UbL) molecules to proteins. PTM by UB/UbL proteins affect protein stability, function, localization, as well as modulate chromatin structure. We therefore predicted that Ub/UbL-related genes within ECs that supervise identity can be discovered using a genetic screen. We recently completed a comprehensive *in vivo* transgenic RNAi screen targeting most of the conserved Ub/UbL genes in the fly (~600 genes). We identified 15 prominent Ub/UbL-related regulators of ECs identity. Genetic inactivation of these regulators impairs cell identity, gut homeostasis and integrity, and overall fly survival. Lineage tracing experiments combined with genomic analyses suggest that maintaining identity by these regulators involves the regulation of enhancers activity, chromatin dynamics, and high order nuclear organization

A general model for supervising cell identity. The emerging role(s) of higher-order chromatin and nuclear organization in the supervision of cell identity is only beginning to unfold (12). The development of powerful genomic techniques has enabled impressive correlative studies and meaningful understanding of the 3D organization of the differentiated nucleus. A simplified model for regulation of the differentiated state is shown in Figure 4; Identity regulators act either directly or via identity transcription factors (TFs) and maintain the differentiated identity by activating the differentiated transcriptional signatures. Concomitantly these transcription factors inhibit non-relevant gene programs of previous and non-relevant fates. Identity TFs may activate or repress the above programs. They also induce the expression of fate determining TFs, terminal TFs or maintenance TFs that together with transcriptional co-factors form the identity TF network. In part, maintaining identity programs involves the precise regulation of enhancers. In parallel, a repressive arm of cell identity involves the silencing non-relevant gene programs in the vicinity of LADs. This involves heterochromatin (HC) inducing modifying enzymes such as H3Kme2/3 methylases, as well as nuclear lamin and other lamina-related proteins. Together these supervisors anchor and maintain HC to the nuclear periphery. Both arms of cell identity regulation must be continuously in place to shape the unique nuclear organization of the differentiated cell safeguarding its identity (12).

The current challenge of our lab is to unfold the regulatory ubiquitin network the supervise cell identity, and to translate these fundamental concepts to the benefit of cancer diagnosis and therapy.

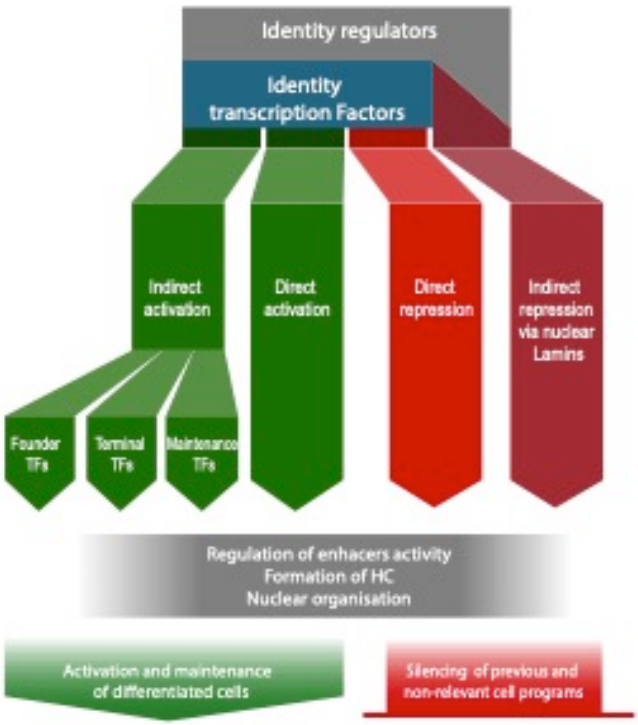

Figure 4: *maintaining the differentiated identity requires continues supervision* (Adopted from 12).

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