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Hylonome

Perspective Article

Viewing problems in bone biology from the perspective of lineage identification

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Just as the field of hematology made major strides in understanding diseases of blood when they migrated from the blood smear and hematocrit to molecular phenotyping mapped to defined stages of progenitor development, so too must bone biologists begin to move beyond standard histology and bone mass measurements to methods that allow cells at defined levels of progenitor and mature development to be identified, isolated and characterized. We are developing a strategy to meet this goal utilizing GFP-based reporters linked to promoters that activate at defined levels of development within the bone lineage. Table 1 indicates the transgenic reporter constructs currently available.

The key to the use of this strategy is demonstrating that the cells identified by a GFP signal represent a cell with reproducible properties that biologists agree meet a defined level of development. With the caveat that an expression pattern is associated with a specific (and commonly shared) transgenic line, an experience can be assembled that increasingly gives confidence in how to interpret the stage of development of a cell identified as GFP+. We have focused on four criteria to make an assignment: (1) the expression pattern in primary bone cell culture (spatial and temporal); (2) bone histology using a tape transfer protocol that preserves GFP in cryosections of bone (spatial association with standard techniques of bone histology); (3) microarray of FACS isolated GFP+ populations and (4) transplantation to assess the differentiation potential of a progenitor population.

Figure 1 illustrates some of the spatial features that associate GFP+ cells to a level of cellular differentiation. pOBCol3.6GFP is expressed in a wide variety of type I collagen producing cells and in bone cell cultures it is first observed in pre-osteoblastic cells in association with AP expression. In bone histology it is seen in periosteal fibroblasts and on bone surfaces that may or may not be associated with dynamic labeling (Figure 2A). In contrast, pOBCol2.3GFP is limited to bone nodules in culture. In bone histology it is always associated with an active labeling surface (Figure 2B) and continues to be active in osteocytes. hOC-GFP and DMP-GFP appear to split the function of pOBCol2.3 into active forming surfaces (hOC-GFP) and osteocytes (DMP-GFP). Figure 3 conceptu-

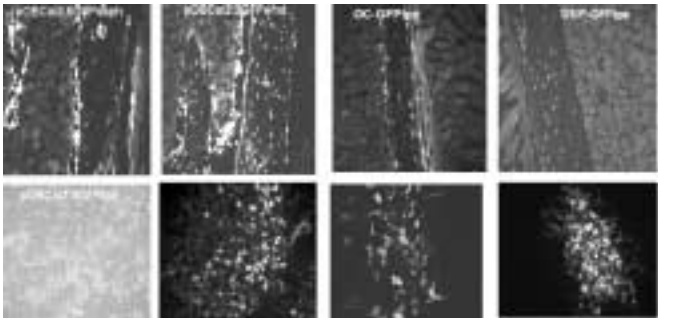

Figure 1. Spatial patterns of GFP expression in bone histology and primary osteoblast cultures (colored figures are available at http://skeletalbiology.uchc.edu/ and go to the image center).

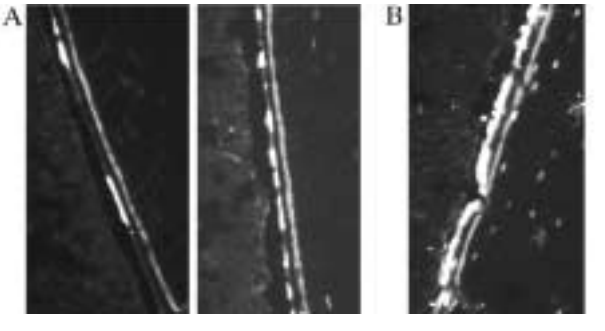

Figure 2. A. Examples of inconsistent co-localization of pOBCol3.6GFP and xylenol orange (XO) labeling. **B.** Co-localization of XO and pOBCol2.3GFP (colored figures are available at http://skeletalbiology.uchc.edu/ and go to the image center).

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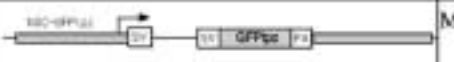
Name	Construct	Identifies:	Status, Use or Source
Type I Col 3.6 pOBCol3.6GFPcyan		Preosteoblasts and early osteoblasts	Saph, Cyan and dsRedII mice will complement GFPtpz.
Type I Col 2.3 pOBCol2.3GFPsaph		Mature osteoblast and osteocytes	Will replace pOBCol2.3GFPemd as a marker of host osteoblasts.
Osteocalcin (human) hOC-GFPtpz		Mature osteoblasts	Mice in use for multiplexed analysis of differentiation
Dental Matrix Protein DMP-GFPtpz		Osteocytes	Mice in use for multiplexed analysis of differentiation

Table 1. GFP reporter constructs for staging osteoblast differentiation in culture and in bone histology (colored figures are available at http://skeletalbiology.uchc.edu/ and go to the image center).

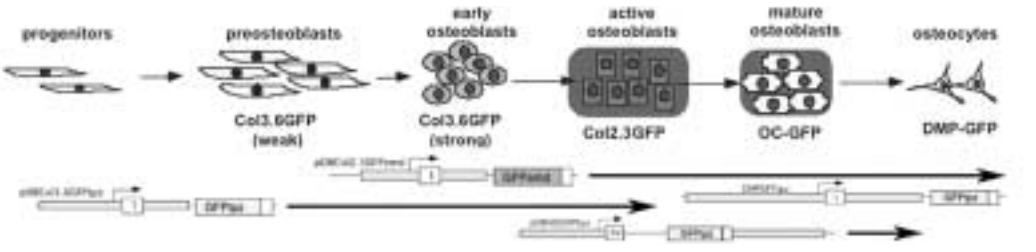


Figure 3. Model of lineage progression as identified by the GFP reporter constructs (colored figures are available at http://skeletalbiology.uchc.edu/ and go to the image center).

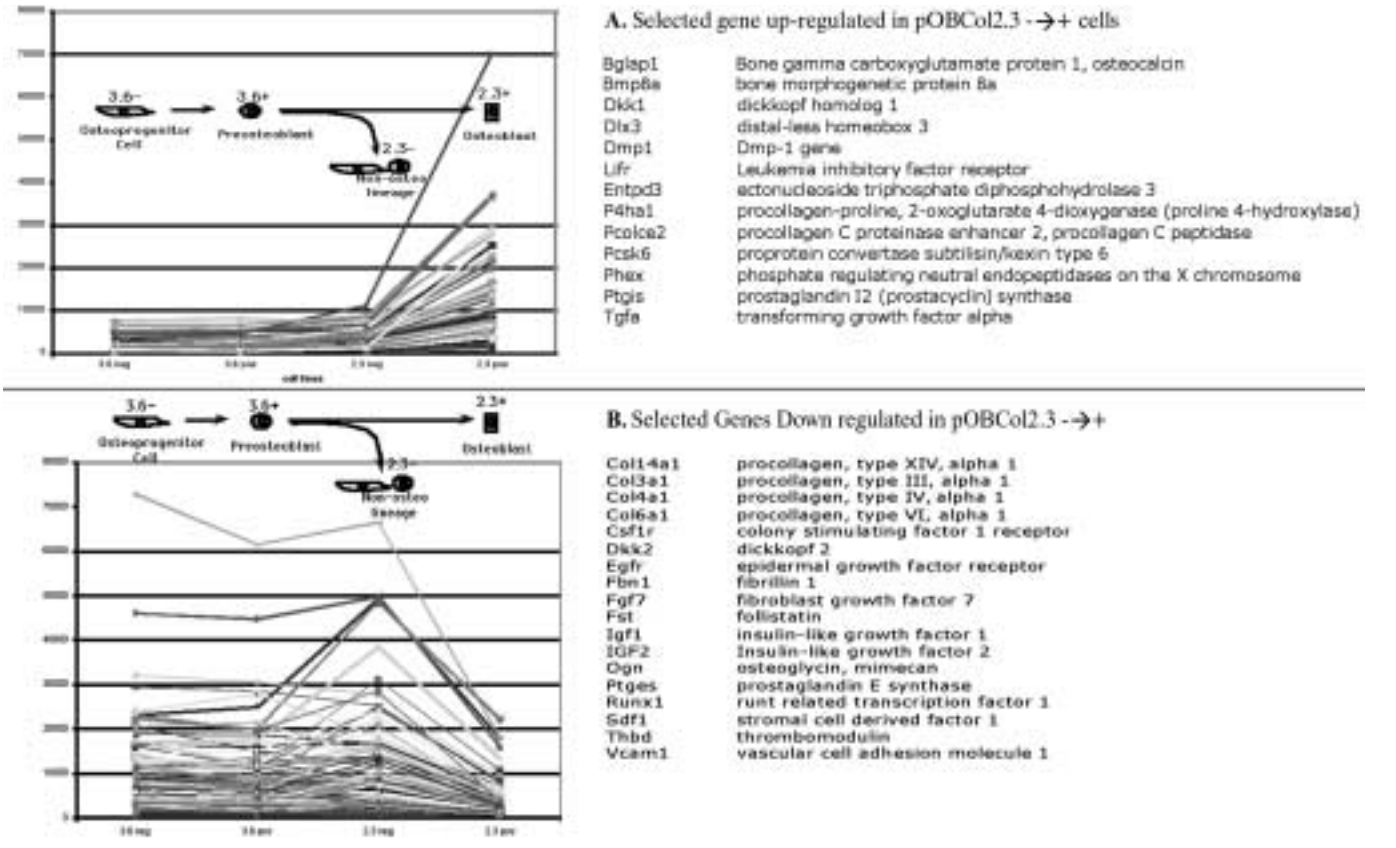


Figure 4. A. Selected gene up-regulated in pOBCol2.3 - → + cells **B.** Selected genes down-regulated in pOBCol2.3 - → + (colored figures are available at http://skeletalbiology.uchc.edu/ and go to the image center)

alizes our interpretation of the expression patterns of each marker transgene.

Microarray has the potential to understand the concerted action of a cell or tissue. However, the interpretation of a regulated gene list can be clouded when the genes are widely expressed and the sample is composed of many cell types or levels of differentiation. The GFP strategy has the potential to overcome this shortcoming of microarray by providing a well-defined and relatively homogeneous sample by FACS sorting. Figure 4A shows that the expected up regulation of gene associated with osteoblast differentiation is found in the FAC sorted pOBCol2.3GFP+ population. Figure 4B shows another cluster of genes that are down regulated in the pOBCol2.3GFP+ population, some of which have been associated with osteoblast differentiation based on RNA samples taken from unsorted populations (e.g., IGF1).

Multiplexing the promoter-GFP colors can increase the power of a histological image of bone. Figure 5 shows the femoral neck region bone from a mouse expressing pOBCol3.6GFPcyan (blue, early osteoblasts) and hOC-GFPtpz (green, mature osteoblasts). Variable degrees of differentiation are seen.

Multiplexing is particularly useful in interpreting a transplantation experiment in which the donor and recipient carry a different color marker driven by the same promoter. Two examples are given. Figure 6 show the result of a host mouse carrying the pOBCol2.3GFPemd gene (mature bone cells) that has been transplanted with total bone marrow from a pOBCol3.6GFPcyan donor mouse. The presence of blue cells on the bone surface of the host bone suggests that circulating bone marrow-derived progenitors have differentiated to bone cells. However, further analysis has shown that the lining cells are early members of the osteoclast lineage (the cells do not deposit a XO label, do not enter bone but do express TRAP) and illustrate that the promoter-reporter transgenes do not always reflect the activity of the endogenous genes. Instead they have to be regarded as a reporter tool that can be associated with a particular cell type. In contrast when the pOBCol3.6GFPcyan donor bone marrow cells are mixed with calvarial derived progenitor cells from a pOBCol2.3GFPemd donor and given to a non-transgenic host by an intramarrow injection, donor derived bone is formed by green cells that do deposit a XO label and do enter bone (Figure 7). The blue bone marrow derived cells remain at the bone surface and do not XO label.

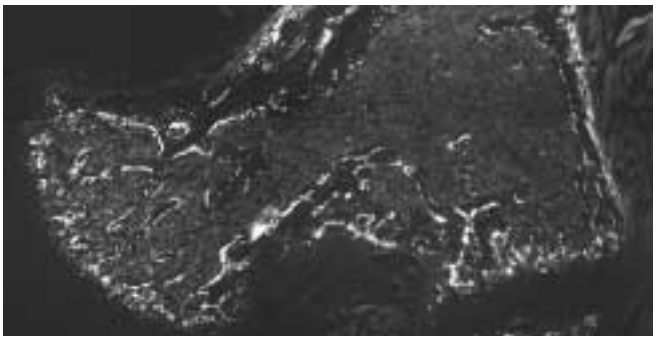


Figure 5. Dual transgene expression shows different levels of osteoblast maturation. The blue cells in the primary spongiosum identify recently differentiated osteoblasts while the green cell of the cortical neck indicate a mature cell population. The transition regions express both colors which appear light blue to white. Equivalent heterogeneity is found in culture (colored figures are available at http://skeletalbiology.uchc.edu/ and go to the image center).

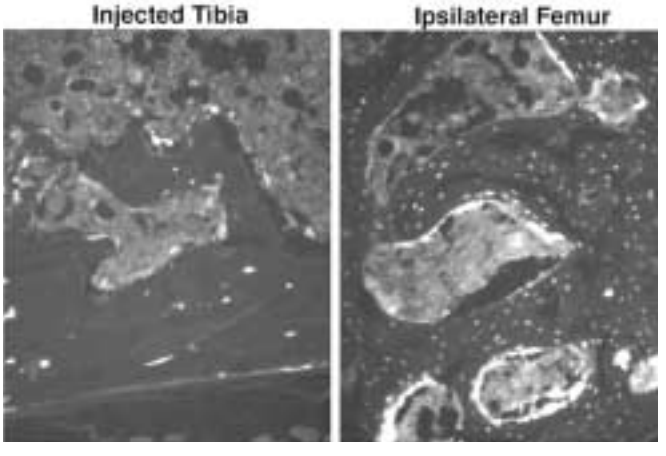


Figure 6. Engraftment by circulating cells from marrow cells of a Col3.6GFPcyan donor (colored figures are available at http://skeletalbiology.uchc.edu/ and go to the image center).

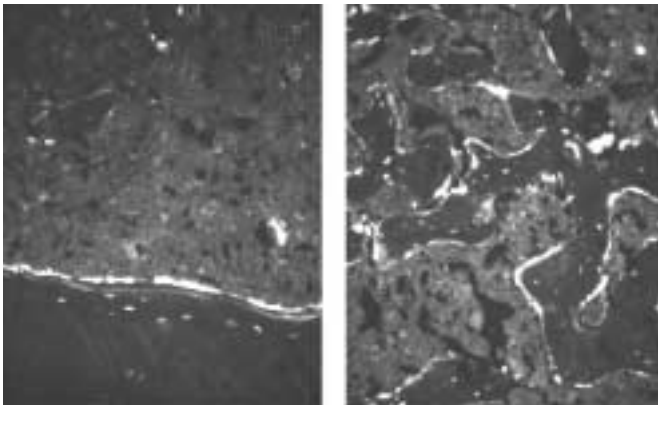


Figure 7. Osteoblast differentiation of donor Col2.3GFPemd cells after intramarrow injection (colored figures are available at http://skeletalbiology.uchc.edu/ and go to the image center).

References

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