In continuation with the previous lecture let us discuss the other artificial markers.

Disadvantage of vital dyes

One problem with vital dyes is that as they become more diluted with each cell division, they become difficult
to detect.

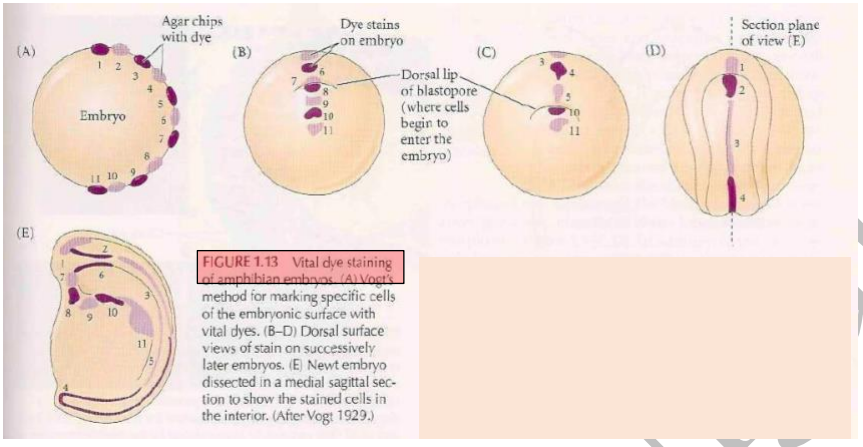
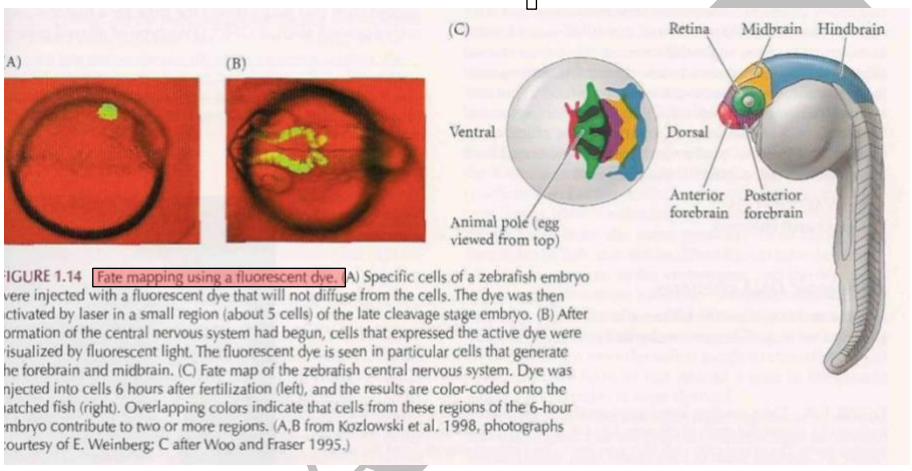


FIGURE 1.13 Vital dye staining of amphibian embryos. (A) Vogt's method for marking specific cells of the embryonic surface with vital dyes. (B–D) Dorsal surface views of stain on successively later embryos. (E) Newt embryo dissected in a medial sagittal section to show the stained cells in the interior. (After Vogt 1929.)



IGURE 1.14 Fate mapping using a fluorescent dye. (A) Specific cells of a zebrafish embryo vere injected with a fluorescent dye that will not diffuse from the cells. The dye was then ctivated by laser in a small region (about 5 cells) of the late cleavage stage embryo. (B) After ormation of the central nervous system had begun, cells that expressed the active dye were isualized by fluorescent light. The fluorescent dye is seen in particular cells that generate he forebrain and midbrain. (C) Fate map of the zebrafish central nervous system. Dye was njected into cells 6 hours after fertilization (left), and the results are color-coded onto the atched fish (right). Overlapping colors indicate that cells from these regions of the 6-hour mbryo contribute to two or more regions. (A,B from Kozlowski et al. 1998, photographs ourtesy of E. Weinberg; C after Woo and Fraser 1995.)

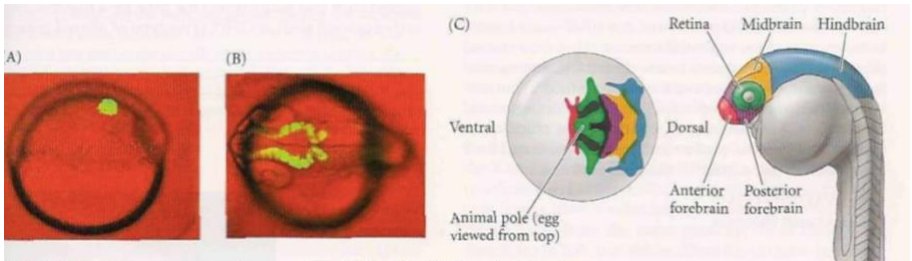
3. **Genetic Markers**

Advantage- they do not spread to neighboring cells and if stably expressed, are inherited by the descendants of the marked cell.

Disadvantage- low efficiency of introducing the gene in the cell.

This method is used to create chimeric embryos by transplanting embryonic pieces from the animals of interest having genetic constitution but similar development pattern. These 'chimeric embryos' are prepared by xenoplastic transplantation using embryos of quail and chick which differ from each other in position of heterochromatin in the cells and cell surface antigens. Retrovirus marking is used to study the fate of embryonic cells by incorporating retrovirus engineered reporter gene into DNA of host cells and once reporter gene is expressed, its gene product is demonstrated by histochemical or fluorescent methods. Retrovirus is the RNA virus that carries a reverse transcriptase enzyme which can make a DNA copy of viral genome of viral genome when it infects the cell. It then integrates in the chromosome of the cell and is passed on to the cell progeny. This results in forming transgenic DNA chimera. Host DNA can also be altered to express green fluorescent protein, GFP which emits green colour. More recently genetic cell labeling technique 'brainbow' has been employed for cell lineage studies. In this the fluorescent proteins are used to tag different proteins in the cells. An array

of colours are generated by different expression of distinct fluorescent proteins. Since this technique was employed to distinguish neurons in the brain, thus it has been termed 'Brainbow'



IGURE 1.14 Fate mapping using a fluorescent dye. (A) Specific cells of a zebrafish embryo vere injected with a fluorescent dye that will not diffuse from the cells. The dye was then ctivated by laser in a small region (about 5 cells) of the late cleavage stage embryo. (B) After ormation of the central nervous system had begun, cells that expressed the active dye were isualized by fluorescent light. The fluorescent dye is seen in particular cells that generate he forebrain and midbrain. (C) Fate map of the zebrafish central nervous system. Dye was njected into cells 6 hours after fertilization (left), and the results are color-coded onto the atched fish (right). Overlapping colors indicate that cells from these regions of the 6-hour mbryo contribute to two or more regions. (A,B from Kozlowski et al. 1998, photographs ourtesy of E. Weinberg; C after Woo and Fraser 1995.)

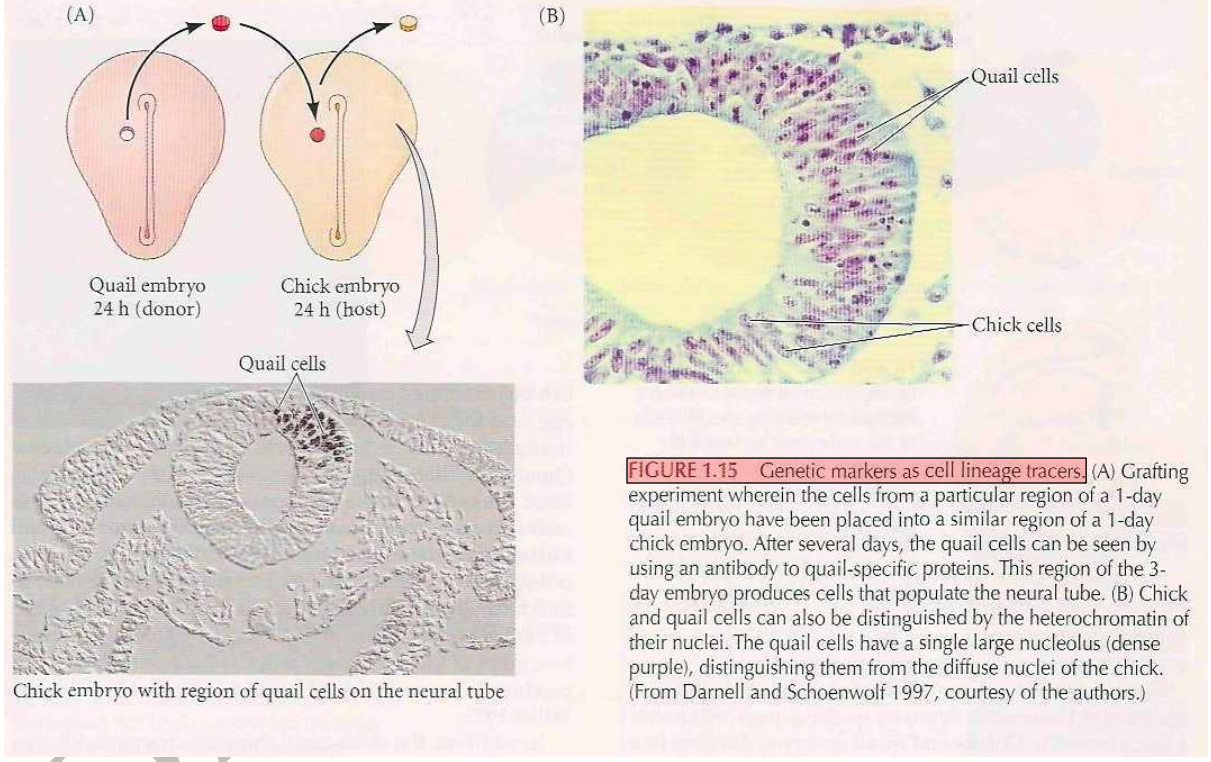
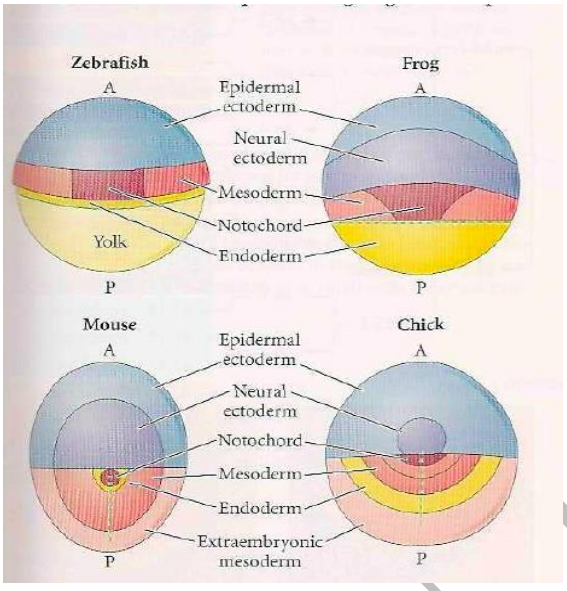


FIGURE 1.15 Genetic markers as cell lineage tracers. (A) Grafting experiment wherein the cells from a particular region of a 1-day quail embryo have been placed into a similar region of a 1-day chick embryo. After several days, the quail cells can be seen by using an antibody to quail-specific proteins. This region of the 3-day embryo produces cells that populate the neural tube. (B) Chick and quail cells can also be distinguished by the heterochromatin of their nuclei. The quail cells have a single large nucleolus (dense purple), distinguishing them from the diffuse nuclei of the chick. (From Darnell and Schoenwolf 1997, courtesy of the authors.)

What is the significance of fate mapping?

Fate mapping can be used to trace cell lineage by following the fate of parts of early embryo during development and to understand the mechanism of morphogenetic movements during gastrulation.



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- Arora R. & Grover A. Developmental Biology: Principles and Concepts. R. Chand & Co.