The source of cells for regeneration

from J.M.W. Slack

A solution to the long standing problem of the source of cells for regeneration has been brought nearer by recent studies on planaria and the amphibian limb. The experiments use two techniques: X radiation doses which can inhibit cell division and regeneration without killing the tissues, and markers which allow the fate of cell populations to be followed.

Both planaria and the limb undergo 'epimorphic' regeneration in which a blastema consisting of cells which are visibly undifferentiated and indistinguishable from one another is formed at the wound site. The blastema cells first proliferate and then differentiate to reform the missing body part containing several different cell types.

The key questions are:— (1) Is the blastema formed (a) from cells drawn from all parts of the body, or (b) from tissues local to the wound? (2) Is the regenerate formed (a) by the *de novo* differentiation of reserve cells, or (b) by dedifferentiation and redifferentiation of functional tissue cells? (3) Is the blastema (a) a mixture of cells pre-committed to differentiate into particular cell types, or (b) composed of similar pluripotent cells able to become any of the cell types in the regenerate?

Although the questions are logically independent the discussion usually becomes polarised into an argument of 'neoblasts *versus* metaplasia'. 'Neoblast' is a term often used to indicate small basophilic cells present in planaria and other organisms, or sometimes in a special sense to indicate mobile pluripotential cells reserved for regeneration. 'Metaplasia' is the dedifferentiation of functional cells followed by redifferentiation of the cells or their progeny into a different histological type — for example transformation from myotube to chondrocyte. The 'neoblast *versus* metaplasia' argument thus corresponds to options 1a, 2a and 3b *versus* options 1b, 2b and 3b. Although the controversy has become fixed in this dichotomy, it must be remembered that other combinations of answers to the three questions are also possible.

J.M.W. Slack is in the Imperial Cancer Research Fund Laboratories, Mill Hill, London.

Planaria can regenerate from either an anterior or a posterior cut surface and contain scattered small basophilic cells which have been called neoblasts by many authors. Early experiments[1] showed that regeneration could be prevented by X-irradiation, but if the worm contained an unirradiated portion (either a shielded region or an unirradiated graft) then it could regenerate fully, even when the healthy portion was some distance from the level of amputation. The time taken for regeneration to begin increases with this distance and correlates with migration of the neoblasts through the intervening tissue.

This showed that a blastema can be formed from migratory cells and is capable of forming all the tissues of the regenerate. However, it did not exclude the possibility that the migratory cells were formed by dedifferentiation, or that in unirradiated worms differentiated tissues local to the wound can also contribute to the blastema. Indeed, histological and electron microscopic studies suggest that local dedifferentiation does occur[2].

Now studies have been published[3–6] on the regeneration of a strain of planaria which are naturally occurring mosaics: the somatic cells are triploid, the cells of the male germ line are diploid and the cells of the female germ line are hexaploid. Regeneration from the gonadless regions gives rise to worms whose somatic tissues consist wholly of triploid cells, whilst regeneration from a cut surface through the gonadal region gives rise to blastemas and regenererates containing some diploid and/or hexaploid cells. Of particular significance is that myotubes with diploid nuclei were found in the muscle of the regerated pharynx[6]. If germ cells and their precursors are accepted as *bona fide* differentiated cells then metaplasia has clearly occurred by the sequence: germ cell→ blastema cell→ muscle cell. However, it could be argued that the germ cells are a special case because in other circumstances they may exhibit pluripotency without passing through a sexual cycle, for example in mammalian teratocarcinoma. This objection might be overcome by building up stocks of worms whose somatic tissues are of an unusual ploidy so that metaplasia between different somatic tissues can be looked for.

Many species of urodele amphibia will regenerate limbs after amputation. The limb contains no small basophilic cells and the histology of the blastema suggests formation by dedifferentiation of muscle, cartilage and connective tissue. Amputation through a small X-irradiated region does not result in regeneration even when the remainder of the limb and the rest of the animal is unirradiated[7] so, in contrast to the planaria, it seems that a blastema cannot be formed without the participation of tissues local to the wound.

Recently studies on metaplasia have been carried out using axolotl tissues labelled by tritiated thymidine and triploidy[8–10] or pigmentation and allogenic differences leading to graft rejection[11,12]. Grafts of a particular marked tissue into an X-irradiated limb followed by amputation of the limb through the graft leads to the formation of a regenerate wholly composed of graft-derived cells, confirmed by the retention of the marker in the various cell types. The irradiated background tissue is necessary because although it does not contribute any cells to the regenerate it does contribute pattern information without which few structures are formed from a small graft[13].

The results are summarised in the Table below. It is clear that there is extensive metaplasia between the different internal tissues, which are all derived from the mesoderm of the embryo. However, epidermis never contributes to the internal tissues and vice versa, indicating the existence of a lineage restriction between tissues of different embryological origin.

So although there may be some restrictions on the possible interconversions, there is now good evidence from both planaria and the amphibian limb that some functional cell types can dedifferentiate and that their progeny can appear in the regenerate as different histological types. These results should be taken seriously by those who advocate theories of cellular differentiation based on irreversible events such as the chemical modification or rearrangement of DNA. □

Cell types found in regenerates formed from grafts of particular tissues.

	Grafted Tissue					
	ectodermally derived	mesodermally derived				
cell types in regenerate:	epidermis	dermis	muscle	cartilage	nerve	(sheath)
epidermal	+	—	—	—	—	
myotube	—	+	+	?	+	
chondrocyte	—	+	+	+	+	

+ marked cells are present, — marked cells absent, ? conflict of results between laboratories.

1. Wolff, E. in *Regeneration* (ed. Rudnick, D.) 53-84 (Ronald Press Co., New York, 1962).
2. Hay, E.D. in *The Stability of the Differentiated State* (Springer-Verlag, Berlin/Heidelberg, 1978).
3. Gregmigni, V. & Puccinelli, I. *J. Exp. Zool.* **199**, 57 (1977).
4. Gregmigni, V., Miceli, C. & Puccinelli, I. *J. Embryol. Exp. Morph.* **55**, 53 (1980).
5. Gregmigni, V., Miceli, C. & Picano, E. *J. Embryol. Exp. Morph.* **55**, 65 (1980).
6. Gregmigni, V. & Miceli, C. *Wilhelm Roux's Archives* **188**, 107 (1980).
7. Butler, E.G. & O'Brien, J.P. *Anat. Rec.* **84**, 407 (1942).
8. Steen, T.P. *J. Exp. Zool.* **167**, 49 (1968).
9. Namewirth, M. *Dev. Biol.* **41**, 42 (1974).
10. Dunis, D.A. & Namenwirth, M. *Dev. Biol.* **36**, 97 (1977).
11. Wallace, H. *J. Embryol. Exp. Morph.* **28**, 419 (1972).
12. Maden, M. & Wallance *H. Acta Embryol Exp.* **2**, 77 (1975).
13. Maden, M. *J. Embryol. Exp. Morph.* **50**, 325 (1979).

0028-0836/80/340760-01$01.00