Brief communication

Homeotic regeneration of eye in amphibian tadpoles and its enhancement by vitamin A

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After removal of both the lateral eyes of external gill stage tadpoles of the toad *Bufo melanostictus*, the pineal organ gets transformed into a median eye. This type of transformation occurrs in tadpoles of both control and vitamin A treated groups. However, vitamin A increases the likelihood of homeotic regeneration (57% in the control group and 71% in the vitamin A treated group). Histological studies showed that the newly transformed median eye developed from the pineal organ. The pineal eye so developed possessed all components of a normal eye such as a retina, sensory cells and lens.

1. Introduction

Regeneration is a challenging field for research in developmental biology. During regeneration a lost part of the body regrows. Homeotic regeneration differs from the normal type; it is a clear example of the transformation of one differentiated tissue having a distinctive pattern of metabolic activities to another tissue which is morphologically distinct from the original one and synthesizes a different array of macromolecules.

Lens regeneration too provides an example of such transformation in which previously specialized tissues of the dorsal iris change into other type i.e. lens. The new lens differentiates from a foreign tissue, the iris epithelium, after lensectomy in urodeles and anuran tadpoles (Stone 1959; Yamada 1967a, b; Reyer 1971; Jangir *et al* 1995). Similar observations regarding transdifferentiation or homeotic regeneration in a vertebrate have also been made by Mohanty-Hejmadi *et al* (1992) and Mohanty-Hejmadi and Mahapatra (1994) in their work on amphibian tadpoles. They observed that following amputation legs regenerated from the tail blastema under the influence of vitamin A. Maden (1993) confirmed these findings by using the European frog *Rana temporia*, and gave further evidence of homeotic transformation of supernumerary limbs at the site of tail amputation under the influence of vitamin A, a drug whose use was based on the pioneering work of Niazi and colleugues. Okada (1996) has pointed out that these regeneration studies have heralded a new era of investigations on the molecular mechanism of action of vitamin A. When combined with an exploration of the activities of homeotic genes such studies are expected to contribute to an understanding of positional value in development in terms of gene expression.

The above observations motivated the present study on the homeotic transformation exhibited during regeneration by the tadpole.

2. Materials and methods

The experiments were carried out on tadpoles of initial external gill stage of the toad *Bufo melanostictus*. The animals were amputated after anaesthetizing them in a 1 : 14000 solution of MS222 (ethyl-m-aminobenzoate-methane sulphonate, Sandoz) in tap water. This strength of MS222 solution narcotizes the toad tadpoles within a few minutes and the animals are revived in 5–10 min. A fine oblique cut was made that removed the anterior-most part of the brain including both optic vesicles, keeping the oral armature intact. The vitamin preparation used was

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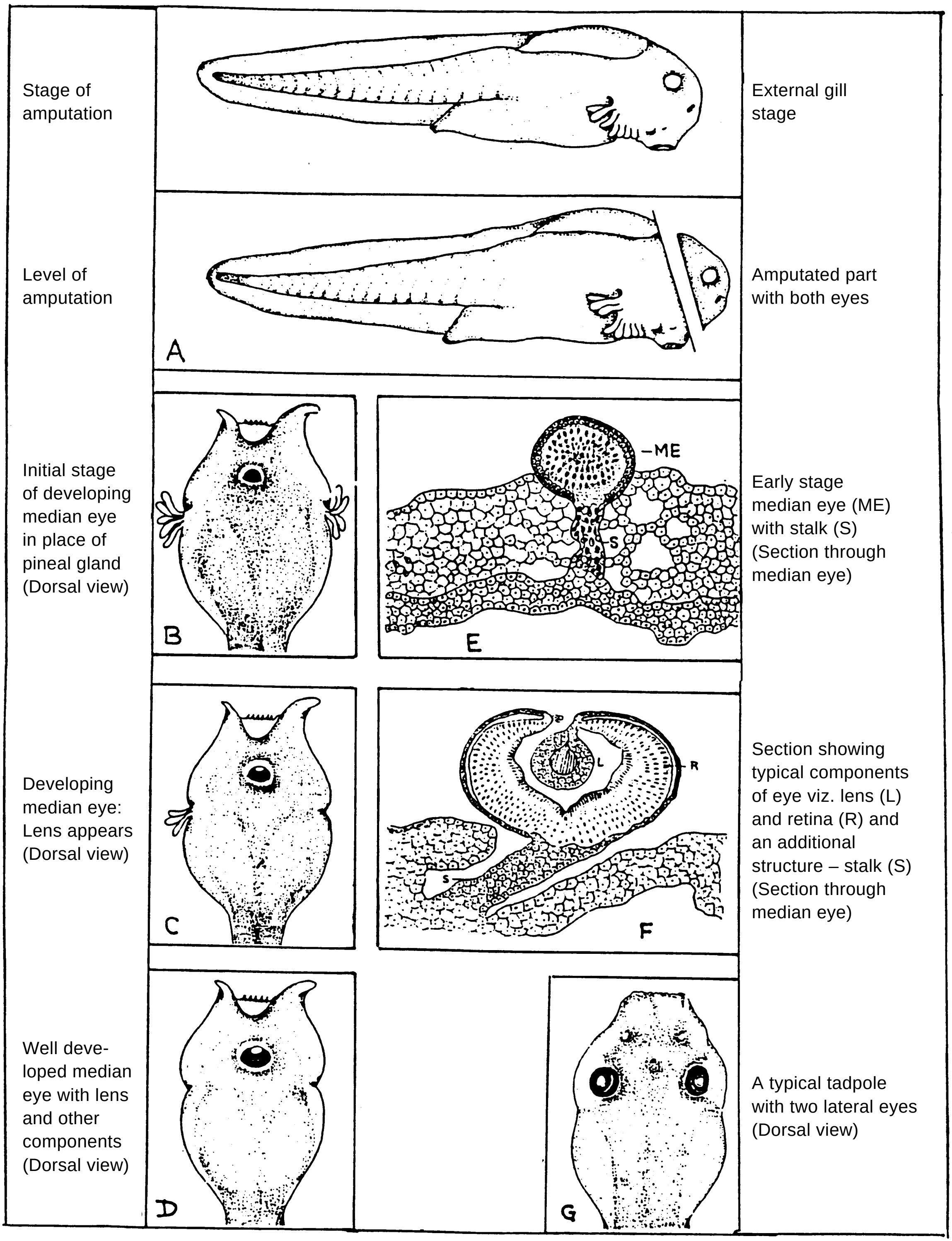


Figure 1. Line sketch diagram shows the level of amputation. The tadpoles were anaesthetized in 1 : 4000 MS 222 solution before operation. Under anaesthetized condition a fine oblique cut was made that removed the anterior most part of the brain including both optic vesicle keeping the oral armature intact. (**A**) Operated tadpoles were reared in tap water and vitamin A solution. Following operation tadpoles were preserved in Bouin's solution at different intervals for morphological and histological studies. (**B, C** and **D**) Developmental stages of median eye. (**E** and **F**) Histological sections of differentiating median eye. (**G**) Normal development of two dorsolateral eyes in normal tadpole.

Table 1. Development of median third eye in control and vitamin A treated tadpoles.

Time of preservation after operation	Number						Median eye/ protuberance (%)	Fused eyes (%)
	Preserved tadpoles after operation	Transdifferentiated median eye			Two frontal eyes/ protuberance	Undifferentiated structure (protuberance)		
		Median protuberance	Median eye	Fused eye				
Control group								
6 h	20	8	0	0	0	12	40	0
Day 1	15	2	8	0	0	5	66·7	0
Day 3	15	0	10	1	0	4	66·7	6·6
Day 7	20	0	12	0	0	8	60	0
Vitamin A treated group								
6 h	20	16	0	0	0	4	80	0
Day 1	15	2	10	3	0	0	80	20
Day 3	15	0	13	2	0	0	86·7	13·3
Day 7	20	0	18	1	1	0	90	5

vitamin A palmitate (Arovit drops, Roche, India). The vitamin was administered by rearing the tadpoles in 15 IU/ml solution of this vitamin. The experiments were carried out at room temperature (32°C–36°C). The method is indicated in figure 1. In all 140 tadpoles were used.

Half of the operated tadpoles were reared in tap water and the remaining half in a 15 IU/ml vitamin A solution. Following operation, the tadpoles were preserved in Bouin's solution at different intervals for morphological and histological studies. An experiment was terminated on day 7 after the operation.

3. Results

Our findings are as follows (see table 1). After amputation of both eyes of external gill stage tadpoles, protuberances appeared in both control as well as vitamin A treated tadpoles. These protuberances later developed into median eyes (figures 2–4). A complete median eye developed at the site of the brow spot in a normal tadpole. Median eyes developed in 40 out of 70 cases of the control group and in 59 out of 70 in the vitamin A treated group. Fused median eyes developed in 1 tadpole of the control group and in 6 tadpoles of the vitamin A treated group (figure 6). In these fused median eyes two complete lenses developed in place of a single lens. In one case the structure so developed appeared like a fusion of two eyes. That is, all the components of the eye developed separately and fused together. Such fused eyes were found only in vitamin A treated tadpoles. Histologically, the newly transformed eyes were found to be similar in all respects to normal eyes in that they contained the components of the normal eye like cornea, lens and retina.

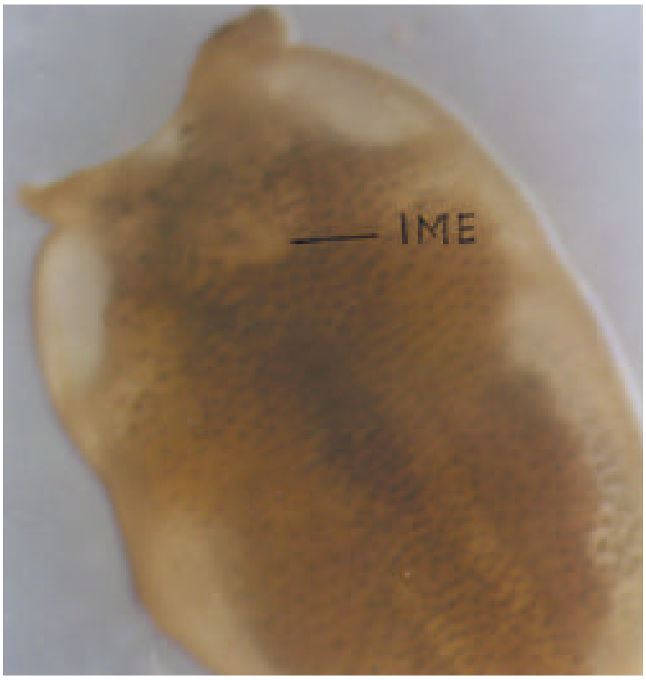
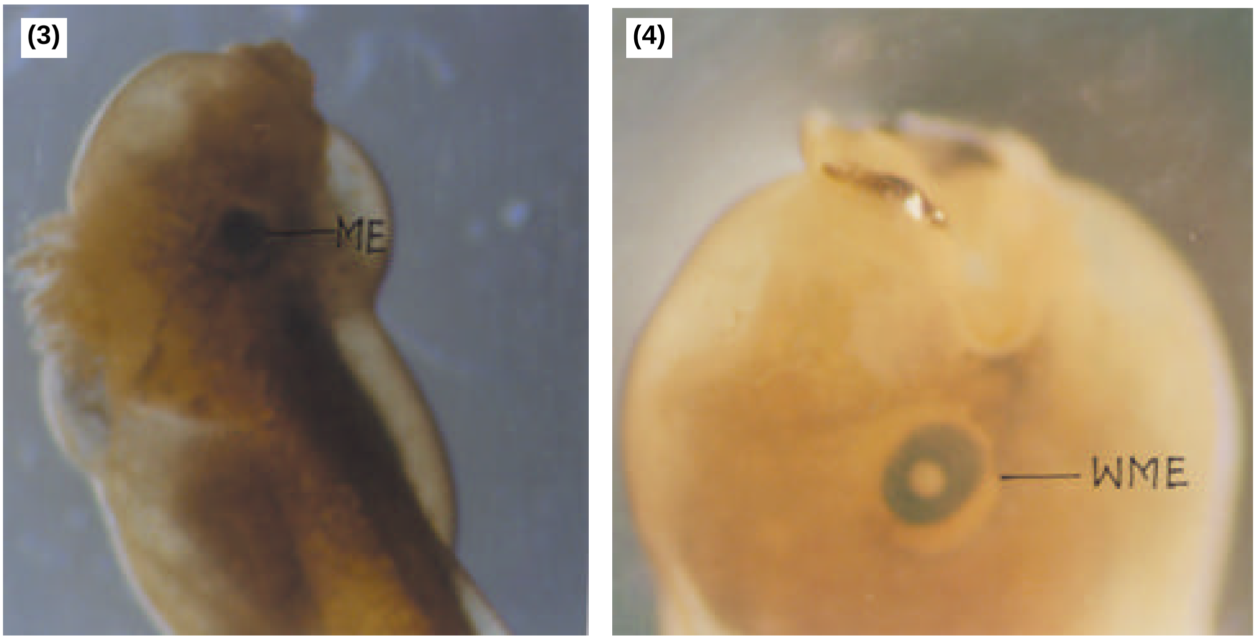


Figure 2. A protuberance like structure appeared in the centre of cephalic region (IME, initial median eye).

The median eyes are of normal size with spacious vitreous chambers. The iris is well differentiated and the lens contains crystalline fibers. A spacious aqueous chamber is present bounded externally by the cornea. The neural retina is also well differentiated. The ganglion cells are arranged in a single row on the inner margins. The ganglion layer is separated from inner nuclear layer by an inner plexiform layer. The visual cells are also differentiated. The transformed organ would seem to be as good as



Figures 3 and 4. These show the further development of protuberance into a median eye. The newly developed median third eye contained cornea, retina and lens (ME, median eye; WME, well developed median eye).

the functional eye. In a few cases a stalked median eye developed (figure 5). It shows connections of the optic stalk to the cells of anterior-most part of the amputated brain.

4. Discussion

The findings are as yet preliminary. The causal chain behind transdifferentiation of pineal gland into an eye is not yet clear. Such development of a median eye has not been reported in late stage tadpoles. In the present study the homeotic eyes so developed are quite normal in structure i.e. eyes are similar to that of normal intact eyes. Histological structures like cornea, iris, retina and lens are also similar to that of normal eyes. Animals with a median eye show normal behaviour when the food material is provided to them. It can be presumed that functionally the regenerated eyes are normal.

Primitive vertebrates are believed to have possessed median parietal and pineal eyes which degenerated in the course of evolution, with the lateral paired eyes acquiring dominance in visual function. Vestiges of these median eyes still persist in various forms in several living vertebrates including some fishes and amphibians and several lizards. Lampreys have a pineal eye possessing even a retina, sensory cells and a flattened lens and recently a living marine fish was reported to have a pineal eye with almost all components of the normal eye (Srivastava and Srivastava 1998).

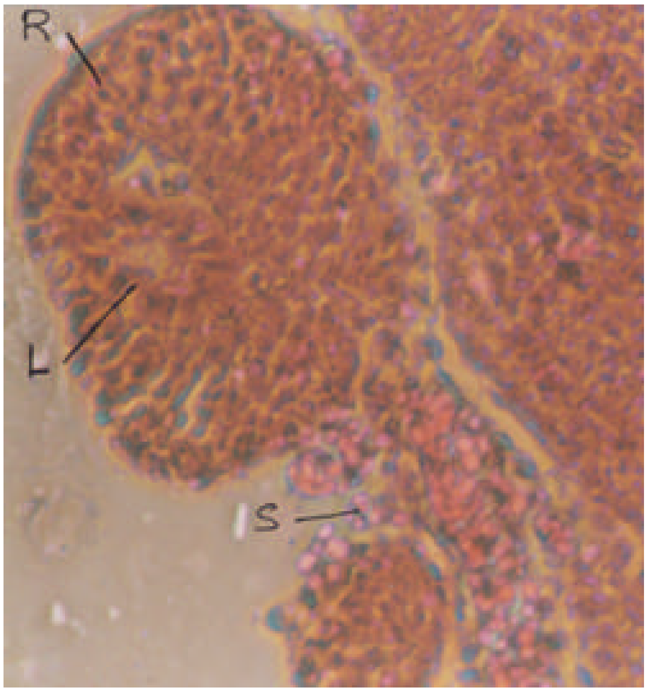


Figure 5. Histological section showing the different components of median third eye (R, retina; L, lens; S, stalk).

In the present study, we have shown that tadpoles of *B. melanostictus* at the external gill stage are capable of replacing normal eyes by means of transdifferentiation of the pineal gland into a median eye. Vitamin A enhanced the

Figure 6. Tadpole showing fused median eye (FME).

percentage of transdifferentiation as well as the formation of fused median eyes. These eyes were quite normal. In some cases two stalked eyes also developed in place of the pineal organ in amputated vitamin A-treated tadpoles.

Histological study reveals that the pineal sacs developed by evagination of the diencephalic roof. The ciliated cells and supporting cells of the pineal gland became specialized as photoreceptors. They show the same general plan as the paired eyes, but with no differentiated lens of the dioptric apparatus. In recent years melatonin binding sites have been demonstrated in the cells of pineal gland as well as in retinal cells and photoreceptors of retina. It has been observed that pinealocytes of the pineal gland share common components of signal transduction (Okada 1991). Based on these similarities, it can be speculated that removal of both the lateral eyes might cause the pineal gland to transform into a complete median eye. Alternatively, some other cells of this region might induce the epidermal cells (ectodermal cells) to transdifferentiate into a median eye. It may be possible that mesenchymal cells of this cephalic region, particularly the eye field region, might induce the epidermal cells to transform into a complete median eye.

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