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THE ROLE OF PROMOTERS AND ADENOVIRAL CONSTRUCTS ON PERSISTENCE OF GENE EXPRESSION IN ORGAN OF CORTI AND MACULAR CULTURES

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Purpose: The utility of adenoviral vectors for studying gene transfer in the cochlea has been demonstrated in numerous studies. Most studies have used a first generation E1/E3 deleted vector with the transgene driven by a CMV promoter and have suggested that gene expression using this system lasts only a few days. Using a luciferase expression system, a variety of promoters and adenoviral constructs were tested on postnatal rat organ of Corti cultures and adult mouse utricular cultures. Intensity of luciferase expression was used to determine the promoter and vector combination that maximized gene expression in terms of degree and duration. Using a green fluorescent protein expression system the distribution of vector within the organ of Corti and macular cultures was determined. The data suggest that robust gene expression can be maintained in auditory neuroepithelium using a variety of promoters. The potential to deliver genes to hair cells as well as damaged neuroepithelium was demonstrated by treating cultures that had been pretreated with aminoglycosides. By varying the promoters used, low to high level expression can be achieved for varying time periods. This combined with the observation that adenovirus can be concentrated into a small volume makes this vector ideal for auditory and vestibular applications.

Introduction: Adenovirus has emerged as an attractive vectoring system for gene therapy over the past decade. Multiply deficient virus vectors with the capacity to carry 10 to 11 KB of foreign DNA have been built and the utilization of these vectors in the clinic and research settings has proceeded at a rapid pace. Several modified adenovectors have been developed that delete sets of early regions that express adenoviral proteins shown to modify the host cell (in particular E1A, E1B and E4). In these studies we have evaluated adenovectors deleted of E1A, E1B, and E3 (GV10) and vectors deleted of E1A, E1B, E3 and E4 (GV11).

Materials and Methods:

- Cultures: Adult utricles or P3 rat cochleae were cultured on Millicel membranes in DMEM supplemented with N1 and glucose. Cultures were maintained under standard conditions and medium changed every three days.
- Luciferase Assay: To determine luciferase expression the entire explant was directly extracted in reporter lysis buffer, the amount of total protein determined by Bio-Rad protein assay and the amount of luciferase activity determined by luminescence and expressed as relative light units per microgram of total protein.

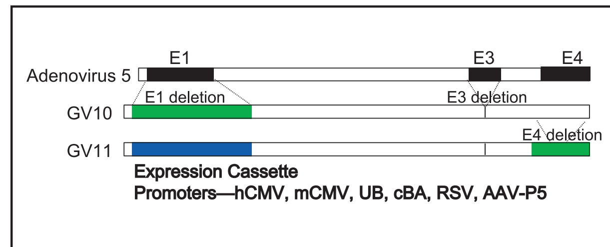

Figure 1. Schematic of adenovirus vectors were constructed and produced as previously described.

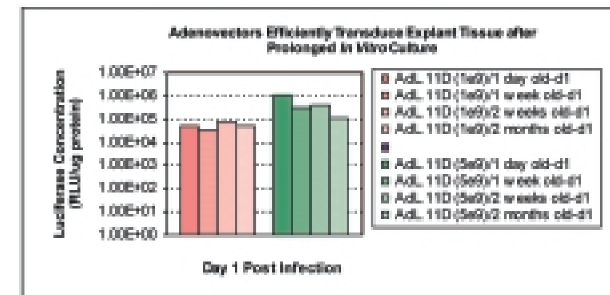

Figure 2. P3 organ of Corti explants were infected after 1 day to 2 months in culture and the level of luciferase trangene expression determined 1 day post infection. These data suggest that there may be subtle changes over time in culture that may affect infectability at high doses of vector. Some published studies have suggested that P3 organ of Corti explants can mature *in vitro* over time, which could lead to these potential differences.

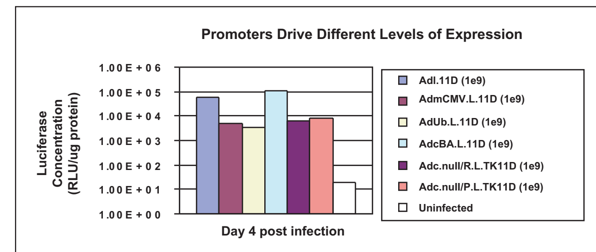

Figure 3. P3 organ of Corti explants were infected with GV11 vectors that express luciferase from different promoters (1e9 particle dose). At 4 days post infection the cultures were evaluated for the level of transgene expression. The highest level of expression was seen with the human CMV promoter (AdL.11D) and cBA promoter (AdcBA.L.11D).

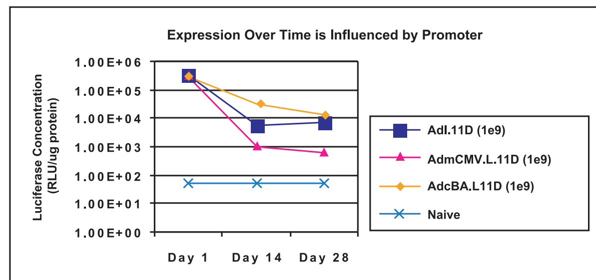

Figure 4. P3 organ of Corti explants were infected with GV11 vectors that express luciferase from different promoters (1e9 pu dose) and the amount of trangene expression followed over time. Luciferase expression from all three promoters drops from 1 day to two weeks and is then relatively the same from 2 weeks to 4 weeks. Interestingly, the mouse CMV promoter shows a very dramatic reduction of expression over time.

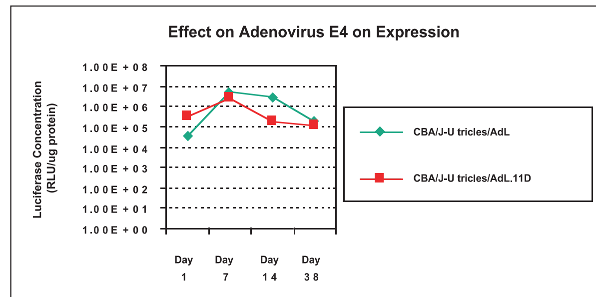

Figure 5. Adult CBA/J mouse macular explants were infected with AdL and AdL.11D. Luciferase expression was monitored over time and found to be similar for both vectors at the dose administered (1e9 pu).

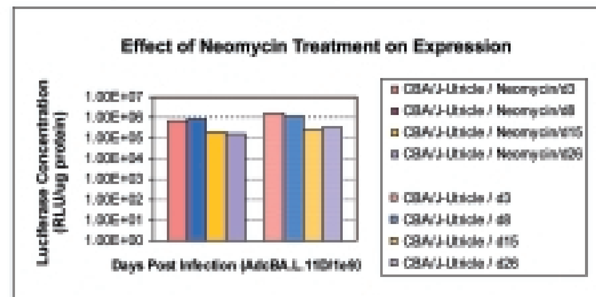

Figure 6. Adult CBA/J adult mouse macular explants were maintained *in vitro* with and without neomycin and vector expression followed over time. No differences in expression were observed after neomycin treatment of explant cultures.

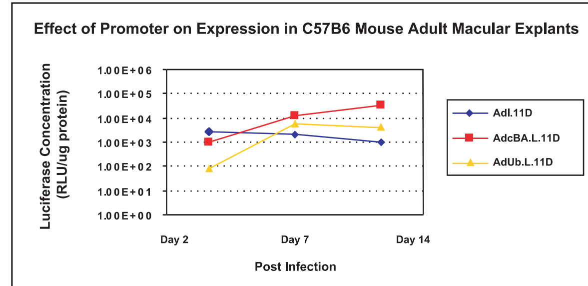

Figure 7. Adult C57B6 mouse macular explants were infected with GV11 vectors that express luciferase from different promoters (1e9 pu dose) and the amount of transgene expression followed over time. Contrary to the rat neonatal explant data expression maintained a steady state or increased dependent on promoter.

Expression in adult mouse macular cultures

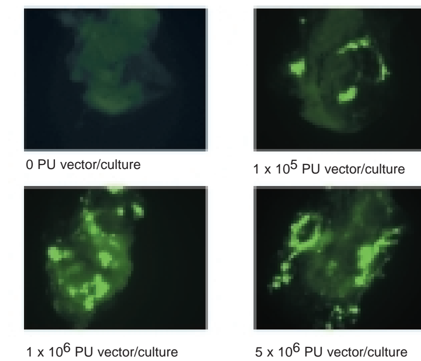

Figure 8. Adult mouse macular cultures were treated with Adf from 1e5 to 5e6 pu and maintained *in vitro*. GFP expression increased with increasing dose of vector. Expression remained stable for 5 weeks and declined to undetectable levels by week 7.

Effect on neomycin pretreatment

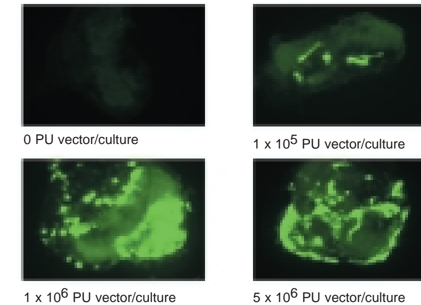

Figure 9. Adult mouse macular cultures were pretreated with 0.1 mM neomycin for 12 hrs and then treated with Adf from 1e5 to 5e6 pu and maintained *in vitro*. There was no statistically significant difference between neomycin and non neomycin treated cultures in terms of number of cells expressing GFP or intensity of GFP expression.

Effect of E4 deletion on GFP expression

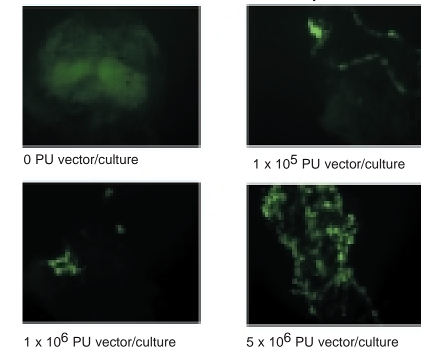

Figure 10. Adult mouse macular cultures were treated with Adf.11D in concentrations of 1e5 to 5e6 pu and maintained *in vitro*. Total number of cells expressing GFP was similar to Adf treated cultures, however GFP expression appeared to be at a lower level/cell. However, expression extended for a longer time period as shown in Figure 11.

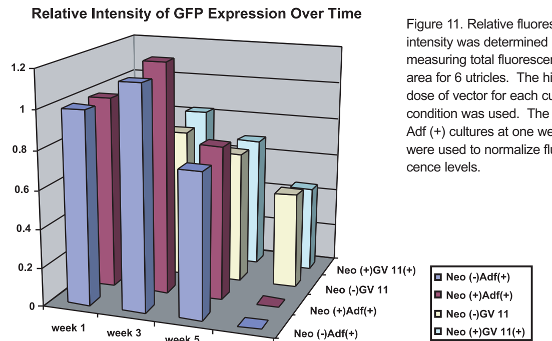

Figure 11. Relative fluorescent intensity was determined by measuring total fluorescence/unit area for 6 utricles. The highest dose of vector for each culture condition was used. The Neo (-) Adf (+) cultures at one week were used to normalize fluorescence levels.

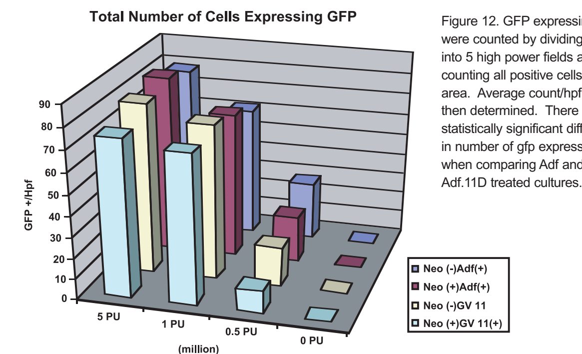

Figure 12. GFP expressing cells were counted by dividing utricles into 5 high power fields and counting all positive cells in each area. Average count/hpf was then determined. There was no statistically significant difference in number of gfp expressing cells when comparing Adf and Adf.11D treated cultures.

Conclusion:

- Promoter choice affects level and duration of gene expression.
- There appear to be differences in level and duration of gene expression in neonatal and adult culture systems.
- E4 deleted vectors are effective and appear to give a more prolonged duration of expression.

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