Sample Exam 2 with Solution

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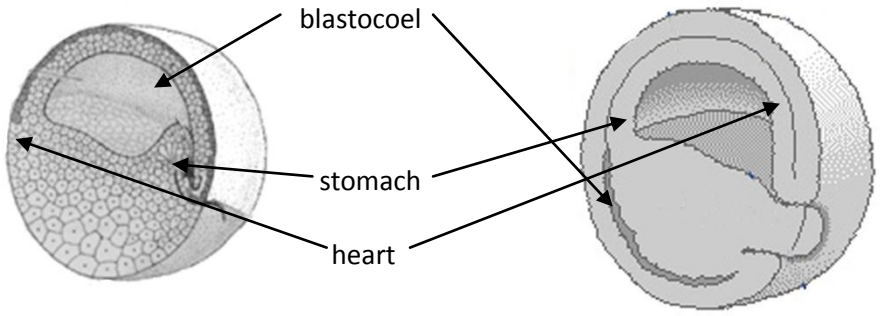
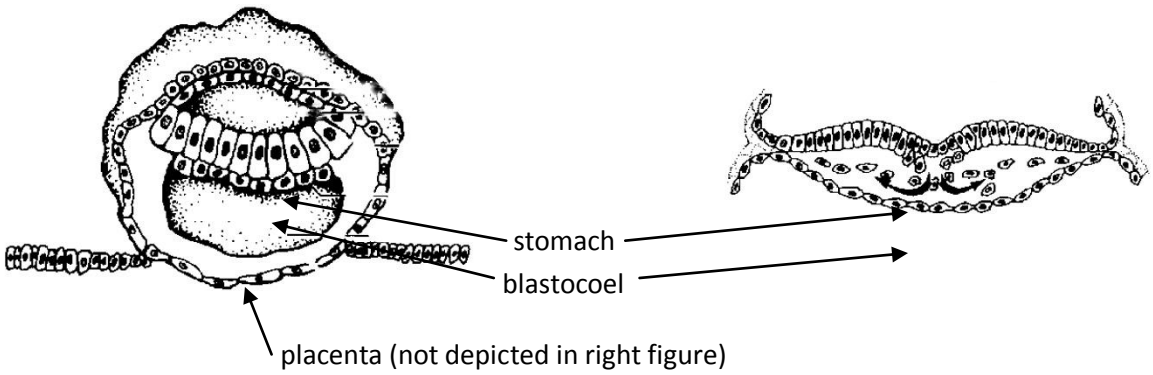
1. a) observe Ca^{++} wave; inject EGTA which chelates Ca^{++}, prevent cortical reaction; inject Ca^{++} or IP_3, induce cortical reaction
   b) cortical reaction; oocyte activation (start cell division)

2. a) iii → iv → v → i → ii
   b) i) ER; ii) cytosol or nucleus; iii) plasma membrane; iv) plasma membrane-associated (cell cortex); v) plasma-membrane associated (cell cortex)

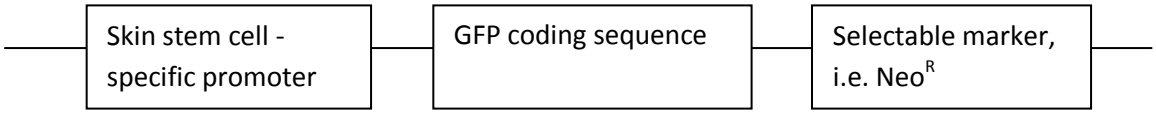
3. a)



   b)



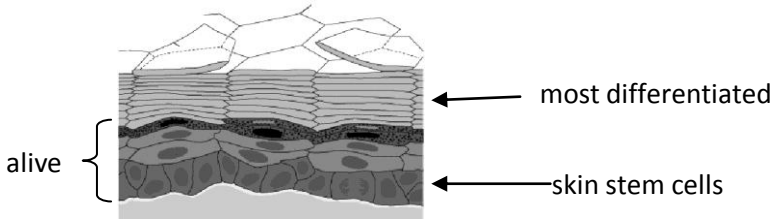
4. a) culture cells with other cells or basal lamina extracellular matrix (to mimic stem cell niche)
   b) treat stem cells with mesoderm inducing factors (i.e. BMPs, activin) or incubate with endoderm
   c) i) 500; ii) 20,000 Northern hybridizations, 1 for each gene; iii) 1 assay using 2 RNA pools (stem cell RNA, skin cell RNA) or 2 assays if doing them separately and later comparing microarray results
   d)



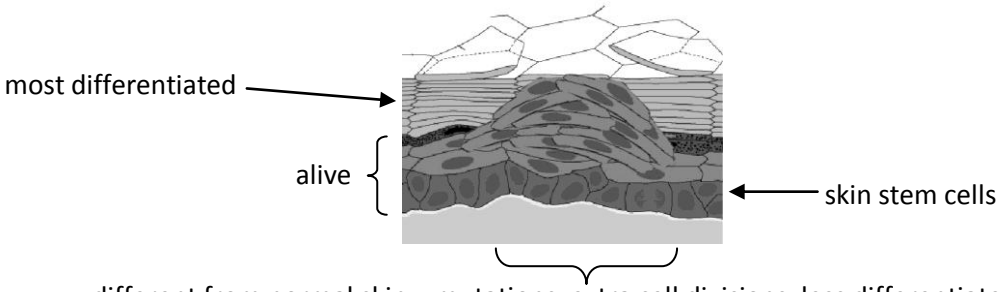
e) transform ES cells, select for proper transformation (antibiotic selection), introduce into blastocoels of embryo, implant into foster mother, find mosaic animals, with germline expression, use offspring
f) purify skin stem cells by using FACS (fluorescence activated cell sorting – skin stem cells should be GFP^+), then assay gene expression (can use microarrays or do Northern blotting)

5. i) perform in situ hybridization (labeled RNA probe complimentary to Wnt11 mRNA) to detect gene transcripts, or perform immunohistochemistry (labeled antibodies that detect Wnt11 protein). For both cases, you should find Wnt11 in the dorsal side – either dorsal mesoderm or vegetal dorsal cells.
ii) inject Wnt11 mRNA or protein into ventral side. If an ectopic dorsal axis is formed, Wnt11 is sufficient to induce dorsal mesoderm.
iii) inject dominant negative receptor (Frazzled) or constitutively active GSK3, if dorsal mesoderm doesn't form then Wnt11 is necessary. Can also perform RNAi to downregulate levels of Wnt11 protein.

6. a)



b) repair of UV-induced DNA damage
c) skin cells are more likely to be exposed to UV
d) mutation in wild-type allele of XP gene (the other XP allele was inherited defective)
e)



different from normal skin – mutations, extra cell divisions, less differentiated