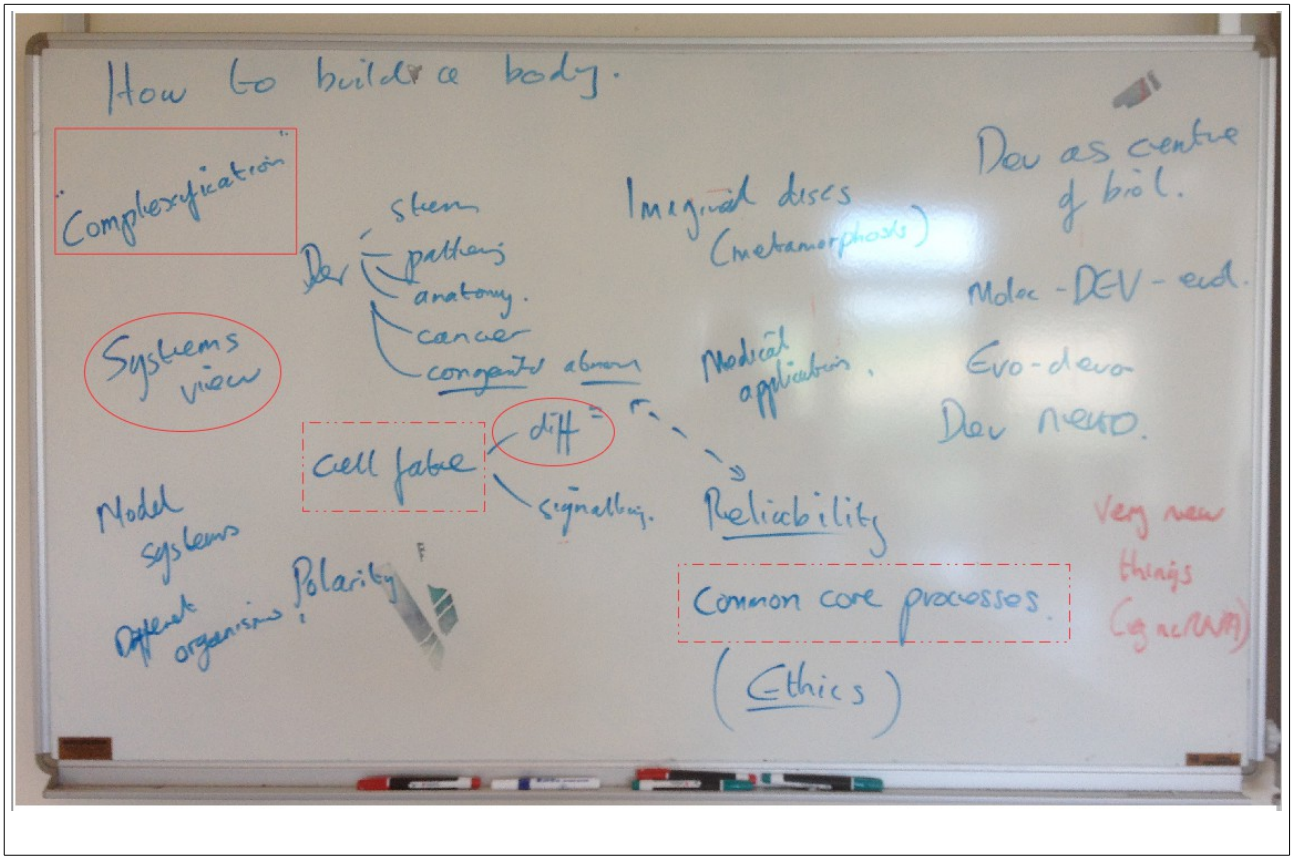
DB4 Cellular Mechanisms seminar notes continued...

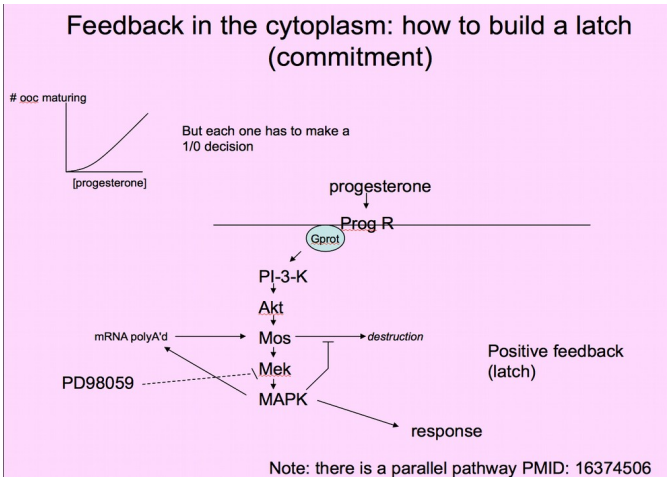
Seminar 4

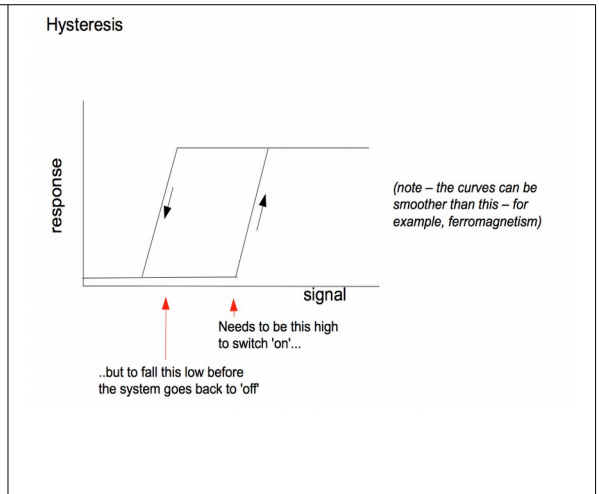
Quick orientation to begin with: here is the 'syllabus' white-board you made at the beginning of the semester, marked with what we have covered already.



This has so far been at the level of genes and things going on in one cell: today we will go up to the level of populations of cells.

First, the homerwork from last week. Here it is drawn out in full, with the feedback loop there. Signals through the system increase the gain of the system, so that it shows hysteresis (switches on at a higher signal than the threshold for switching off: this allows good decision-making in the face of noise).





OK – I think this is probably enough about differentiation and gene control. What I have tried to do, in this section, is to guide your thinking up from the properties of individual genes and their promoters, which are conceptually simple, through small networks of genes, to large genome-sized networks. At this level, we see new properties (feedback, feedforward, the existence of differentiated states, basins of attraction etc) that are not properties of genes, but clearly depend on (**emerge** from) the properties of these individual genes. The point of this was to be able to handle high-level properties of the genome without any mysticism, because you can follow the path from gene actions to genome properties (follow it in that direction, I mean: working back is much harder). The last little bit – the signalling pathway above – was to stress that feedback loops and decision making are not just about events in the nucleus and can be done in the cytoplasm too. They can also be done by cell collectives, and that is why we are moving to patterning next, to exemplify this.

Advice on revision and exam questions for this section (written here because you will try a formative question this week):
Long-answer exam questions are intended to test your understanding. They will usually not be in a form that has one definite right answer, but will instead ask a question that allows you to evaluate two sides of an argument and draw a conclusion. The marks are for the quality of argument: as long as your writing is internally consistent, an argument against the opinions I happen to hold will score just as well as one that agrees with them. Good arguments (in science) usually require three things:

1) A clear statement of premises (including exact meanings of words, if one key word is critical to the question – this applies particularly when a word invented for non-biological purposes is being applied to biology: 'master regulator', 'controller', 'memory', 'stability' etc are all examples. If you say clearly what properties something would have to have to be a 'master regulator', say, then I know the premises of your argument and can follow it and give credit for it, even if your list of properties is different from the list I would choose, provided your list is remotely reasonable).

2) The arguments on EACH side of the question – in biology this would mean making statements backed up with biological examples (and drawing a pathway or process can be much faster that describing it in text: that is fine). You do therefore need to learn some real examples of things – that is the 'rote learning' element of the exam. Just rote learning will never get you beyond a scraped 2/2 though. In general – and this is advice and not a 'rule' – present the side of the argument you will reject first, then the side you will support. That way involves less mental whiplash on the part of the reader when you get to your conclusions section.

3) A clear conclusions section. If you can come down on one side of the argument, do so. If you conclude that we know to little to be able to come to a conclusion, say so and then specify what extra information is needed and why: 'A1' questions will suggest, in outline, what experiment would provide the missing information – if you can do this, you are showing us that you really are becoming a scientist.

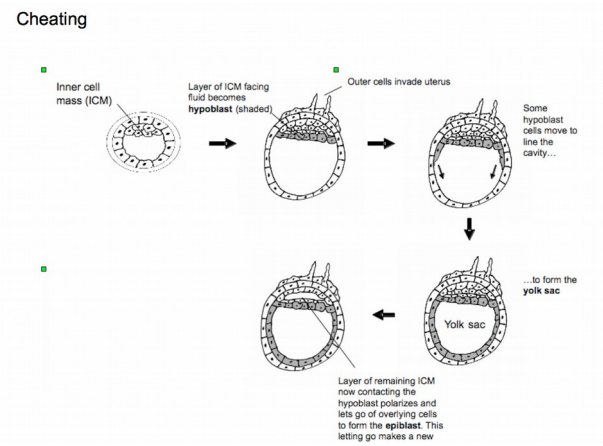
At Honours level, it is important that you structure your essays well and do not simply fire-hose the examiner with randomly-remembered information in the hope that some of it may be relevant. An examiner deluged with 30 bits of apparently random information, 10 of which will be relevant, will give a 3rd or 2/2, whereas an examiner given just 5 pieces of relevant information used really well to build a logical and well-constructed argument and conclusion may give a high 1st. (There is nothing special about the numbers 10 and 5 – I am just using made-up numbers to stress a point).

OK: on to patterning.

We considered patterning de novo: a patternless field of cells pulling itself up by its bootstraps (ie 'bootstrapping itself', or just 'booting up' as the phrase has become in the IT world).

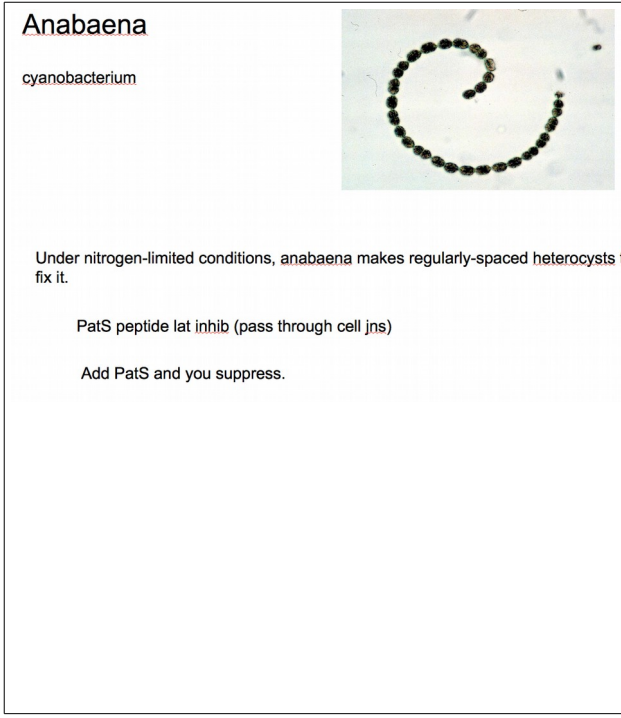
There are 4 main approaches:

- Break symmetry randomly
- Get information from the environment (we did not cover this in class, but I mention it now briefly – see below)
- Lateral inhibition
- Clock-and-wavefront models



Examples of getting information from the environment ("cheating") included using gravity or using free surfaces, for example in the early mammalian embryo (human is sketched to the left: free surface facing blastocoel acts as a patterning cue).

Lateral inhibition is exemplified by Anabaena (http://www.ncbi.nlm.nih.gov/pubmed/11121783)



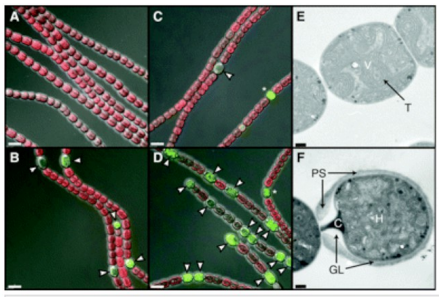
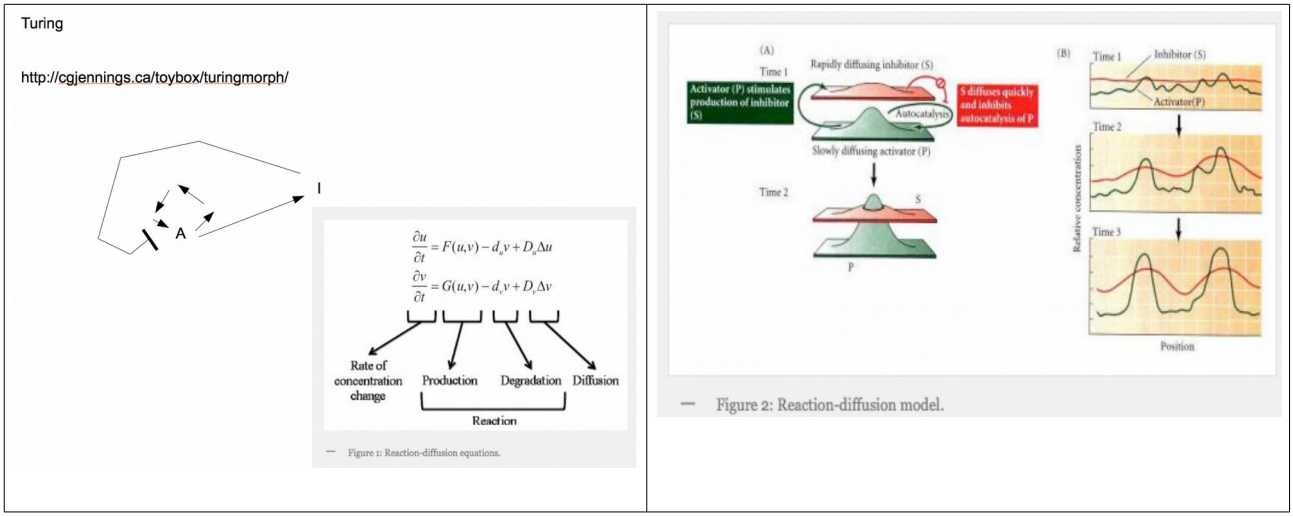


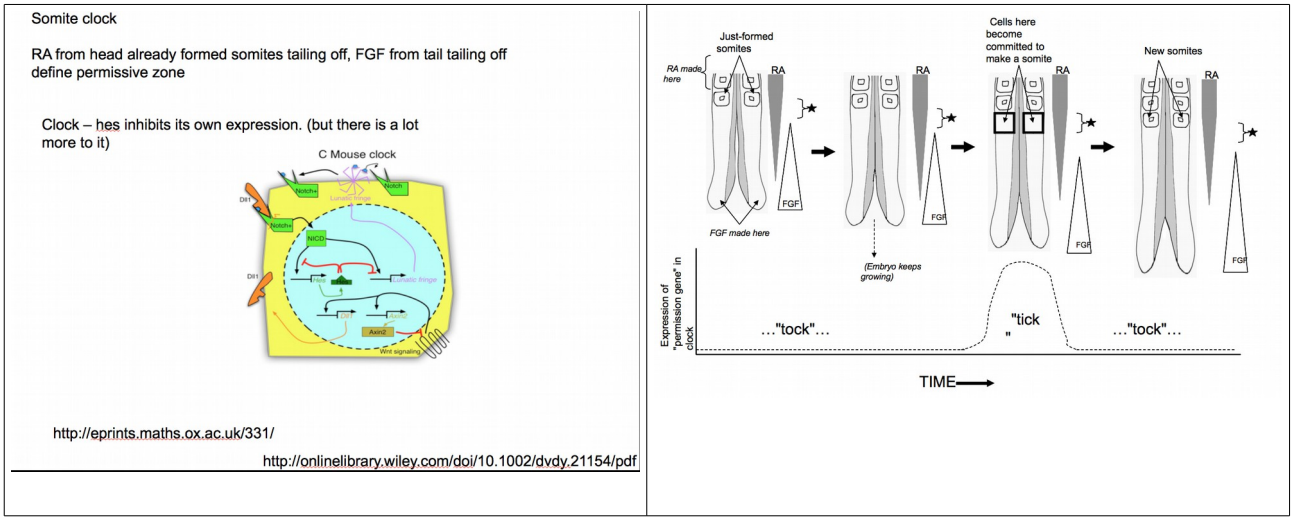
Figure 2.

Heterocyst development in *Anabaena* PCC 7120. Filaments of the wild type carrying a *patS–gfp* reporter grown in medium containing nitrate are composed of vegetative cells (*A*), and have undergone heterocyst development 1 d after transfer to medium without combined nitrogen (*B*). A *patS* mutant strain carrying the same *patS–gfp* reporter grown in media containing nitrate contains a small number of heterocysts (*C*), and 1 d after transfer to medium without combined nitrogen shows a higher than normal frequency of heterocysts and an abnormal developmental pattern (*D*). (*A*, *B*, *C*, *D*) Merged DIC (grayscale), autofluorescence of photosynthetic pigments (red), and *patS–gfp* reporter fluorescence (green) microscopic images; arrowheads indicate heterocysts; asterisks indicate proheterocysts; size bar, 5 μm. (*E*, *F*) Transmission electron micrographs of wild-type vegetative cells (V) and a heterocyst (H) at the end of a filament; T, thylakoid membranes; PS, polysaccharide layer; GL, glycolipid layer; C, polar cyanophycin granule; size bar, 0.2 μm.

This idea can be extended to 2-dimensions: here is Turing's system:



The clock-and-wavefront model used a pattern in time, and a progression in space, to create a pattern in space.



Critical to patterning de novo were combinations of positive and negative feedback, and systems of cell-cell communication.

Finally, we noted that gradient-defined patterns usually form (in animals) at scales of the order 100 micrometers; they cannot be used for very large patterns because the gradient will be too shallow for cells to detect their position reliably (the clock and wavefront model is a nice way of getting round this – move a steep gradient along. Cytonemes and other ways of making cells larger with respect to the gradient are also useful).

'Homework'

"***Noise in biological systems: is it useful to, or a nuisance to, embryonic/foetal development?***" (This question is deliberately chosen so that it addresses something we have touched upon many times already, but about which we have never actually written a white-board).

Before you tackle this week's exercise, let me explain the point of practice questions and the

feedback. Please do carry on to read the next paragraph, because there is strong evidence that most students (and indeed many staff) misunderstand this.

Obviously, since you are unlikely to find this precise question in an exam, the point is *not* that you should apply feedback to your effort to generate a 'perfect essay' for that question and memorize it. Instead, you are trying to improve two skills/ areas of knowledge. The first things to be gained/ improved are the skills concerned with actually writing an essay: these include 'getting to the heart of a question', assembling data and arguments on both sides of it, presenting these in a logical way and reaching a conclusion. The second thing to be gained/improved is very hard to define but very important, not just for exams but for leaving university with the 'deep' attributes of an Honours graduate: it is an appreciation of academic 'quality' and an ability to recognize it and to aim to achieve it.

There is a wide-spread misunderstanding (Sadler 2010, 2013) that 'learning through feedback' will work if you are just given some comments about the work you hand in . This may help with the first set of skills mentioned above but does very little for the second. For feedback to help you with this, it needs to loop round a few more times so that you practice judgement for yourself, as well as have your work judged (Boud et al 1999, Nicol 2010). Therefore, answering this practice question will involve the following feedback loop:

1. Produce an electronic (pdf) answer to the question (no more than 3 typed pages and do not spend more than 30 mins typing it: for this purpose, spend as long as you like preparing. You do not need references as this is a 'mock' exam essay and you would not have them in an exam question. DO NOT put your name or matric number on your essay.
2. Write (/type) about 1 paragraph in which you judge the strengths and weaknesses of your own essay, and judge what grade you honestly expect. This is a private document to you (I will never ask to see it) so please be honest with yourself. Keep this somewhere safe; I'll refer to it as your 'reflective document' from now on.
3. Send me the essay no later than Monday 26th.
4. I will send you everyone's essays (they are anonymous, remember).
5. Read the other essays, and give them a grade: list these grades, with some way to identify which essay they come from (eg the first 6 words of the essay) on the reflective document in a new section under your previous judgement of your own essay.
6. Now look back at your judgement of your own essay: do you still agree or would you alter your grade? Write your thoughts on this briefly in a new section of the reflective document.
7. BRING copies (paper/ electronic) of the essays to Friday morning.
8. I will hand out my own grading and feedback on all of the essays, to everyone (this is still anonymous – nobody will know which essay comes from which person). I will also discuss any general feedback points.
9. (**Do not skip this**): after the class but still on that same day, make a new section on your reflective document and summarize anything you have learned from this process, about how to write an excellent essay, about common problems, *and any way in which your own judgement of quality has changed*. KEEP THIS – it will be useful to you all year.

Please respect the anomymity of this exercise. You may not care about your own privacy, but if some people break their anonymity then they are also reducing it for others, as the number of possible student-essay combinations reduces.

References for the evidence-based teaching (of course you do not have to read these: I provide them simply to assure you that there are good reasons behind the way I have designed your feedback exercise)

Boud D, Cohen R, Simpson J (1999) Peer-learning and assessment. *Assessment and Evolution in*

Higher Education 24: 413-426

Nicol D (2010) From monologue to dialogue: improving written feedback processes in mass higher education. *Assessment and Evolution in Higher Education* 35: 501-517

Sadler R (2010) Beyond feedback: developing student capability in complex appraisal. *Assessment and Evolution in Higher Education* 35: 535-550

Sadler R (2013) Teaching learners to see. In Merry, Price, Carless & Taras (Eds) *Reconceptualising feedback in higher education.* Routledge. pp55-63

Taras M (2013) Feedback about feedback: uncrossing wires across sectors. *Reconceptualising feedback in higher education.* Routledge. pp30-40

Finally, here are some questions from last year's pool:

SHORT ANSWER

Define the term 'emergence', illustrating your definition with two examples drawn from animal development.

- Define the term 'adaptive self-organization' and illustrate it with an example
- There were once three hypotheses about how enhancers are able to act at a disance from the promoter(s) that they regulate; scanning (transcription factros bind enhances then 'walk' along the chromosome to the promoter), looping (intervening DNA loops out to promoter and enhancer complexes touch) and twisting (enhance-binding elements alter the twist of DNA). Illustrate with diagrams the key experiments that eliminated two of these possibilities, indicating clearly which possibility each experiment eliminated. Does eliminating two possibilities prove the veracity of the this? (a single word answer, yes/no, will do for this part)
- Describe/draw an example of a gene whose transcription is controlled by two transcrpition factors in a manner that approximates to (a) a Boolean AND function and (b) a Boolean OR function
- The figure above depicts a 3-element N-K Boolean network: what are its attractors?
- What is the role of positive feedback in development

LONG ANSWER

- The molecular interactions that give rise to large non-living structures, such as crystals, can be explained by the laws of physics and chemistry alone. What principles must be added to physico-chemical mechanisms in order to explain the organization of a bioligical structures such as a cell? Use examples to illustate your arguments.
- Where in the cell does biological control lie?
- Where in the embryo does biological control lie?
- Plan an experiment that could, <u>in principle</u>, disprove the looping model of enhancer action
- Describe how feedback is used to shape biological structures at (a) a subcellular level, (b) the level of cellular interations and (c) the tissue or whole-organism level.
- How appropriate are hierarchical models of gene control in animal development? Justify your answer with examples
- What do N,K Boolean network models suggest about self-organization in development and the evolution of new cell types
- How can cells make clear decisions, without vacillating, in respnse to noisy, fluctating

signals?

- How is cell-cell signalling used in animal development?