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Perspectives

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Unicorns Revisited

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IN 1988, CAIRNS, OVERBAUGH and MILLER claimed to have evidence that some bacterial mutants arise as a specific, adaptive response to the selective environment of the moment, "adaptive" because the mutant has gained the ability to grow, and "specific" because other, irrelevant mutants do not accumulate during the selection. The environment appeared to "direct" the mutational process!

Whatever *you* may think, those who work on "directed" (or "adaptive") mutants seem to believe that the reality of their phenomenon has been securely established. In both hasty (HALL 1992) and responsible (STEELE and JINKS-ROBERTSON 1992) papers, even yeast has been claimed to show it. Thus, quite appropriately, FOSTER and CAIRNS (1992) have moved on to the next phase, testing the models that have been offered to explain "directed mutagenesis."

Since many otherwise intelligent people will doubt the phenomenon until its mechanism has been elucidated, FOSTER and CAIRNS are serving science well by directing their attention to the models. Not that they have identified the mechanism for the origin of directed mutants! That paper is yet to be written. However, FOSTER and CAIRNS have blown away several possibilities with further studies on the "directed" revertants of *lac* mutants.

The first model they go after is the one nobody liked anyway, because it would circumvent the Central Dogma. This model envisioned a feedback from "successful" protein to message that encoded it to gene that encoded the message. An undirected, propitious transcription error might thereby become immortalized as a DNA mutation. The experiment that disposes of this blasphemous model is tidy and convincing. It rests on the simple observation that among the directed revertants of a Lac^- amber are both true revertants, which could, in principle, be directed as described above, and tRNA suppressor mutants, which could not.

The second model challenged proposes that transcription is inherently mutagenic and that the lactose-induced Lac^+ revertants are a consequence of the well known ability of lactose to induce transcription of the *lac* operon. Of course, the tRNA suppressors noted above already stand against this hypothesis. As further evidence, FOSTER and CAIRNS note that Lac^- mutants that are constitutive for transcription of the *lac* operon revert only in the presence of lactose. This experiment falls a bit short because there is no demonstration that the constitutive cells are, in fact, constitutive when they are starved. The argument that transcription plays no important role in the origin of these directed revertants is made more convincing by the demonstration that isopropyl thiogalactoside (IPTG), a gratuitous inducer of *lacZ*, does not by itself cause the accumulation of Lac^+ cells. Furthermore, it cannot be argued that the starved (or semistarved) cells did not respond to the IPTG because the IPTG enhanced the ability of existing Lac^+ cells to yield Lac^+ colonies when the cells were exposed to lactose.

I was pleased to see those models crash. I was less pleased to see my model crash. That model (STAHL 1988) proposed that repair synthesis occurring here and there in stationary phase cells allows mutations. Postreplicational mismatch repair, proposed to act slowly in these cells, eventually repairs any irrelevant mutations. Mutations that allow the cell to escape its metabolic bind, however, lead to chromosome replication with the consequence that the mutation is fixed before it can be repaired. FOSTER and CAIRNS show, with minor caveats, that the postreplicational mismatch repair system as we know it (the Mut system) is not involved in the selective disappearance of irrelevant mutations (and see JAYARAMAN 1992). While they are at it, they demonstrate that selectivity does not depend on the alkylation repair pathway, either.

Where does that leave us? FOSTER and CAIRNS call our attention to the following two observations: (i)

Directed revertants of a Lac$^-$ frameshift mutant arise at a reduced rate in a strain that carries a *recA* allele (CAIRNS and FOSTER 1991; and see JAYARAMAN 1992). (ii) The base change that results in reversion of one of the *lac* mutants studied almost certainly depends on DNA replication for its occurrence. Revertants of this *lac* mutant can be environmentally directed, telling us that DNA replication is required for the origin of some (maybe all) directed mutants. Because these revertants did not accumulate in the absence of lactose, the authors conclude that the replicated DNA is unstable if the cell cannot benefit from it.

These two observations suggest to the authors a model involving gene amplification:

> . . . we can account for all the experimental evidence, at least in our system, with the following hypothesis. In stationary phase, cells may be amplifying limited regions of their genomes. We can imaging (sic) this as simple duplication, which is known to occur at frequencies of 10^{-3} to 10^{-4} per cell (ANDERSON and ROTH 1981), or as more extensive amplification. These extra DNA copies would be inherently unstable, but might have an increased chance of containing errors. The cell that achieves a useful mutation in one of these copies could exit stationary phase, begin to grow, and resolve the amplified region by a RecA-dependent process. This hypothesis predicts that anything that increases the error-rate of DNA synthesis will increase the rate of post-selection mutation, but the process will still be RecA-dependent. RecA could, in fact, be required for each step in this process (LARK and LARK 1979; TLSTY, ALBERTINI and MILLER 1984; DIMPFL and ECHOLS 1989).

Does this model really work? I have quoted the model in full so that you, Dear Reader, can judge whether I am being fair when I say, "Hardly, at least not if 'amplification' means the accumulation of tandem duplications as implied by the citation of ANDERSON and ROTH (1981)."

Although the paragraph quoted is unclear, I suppose that the tandem duplication model works like this: One element of the duplication mutates. If the mutation is irrelevant for cell growth under the selective conditions, there is an even chance that the mutation will be retained when the duplication is lost (as it will be) by some recombinational process ("looping out," unequal crossing over, etc.). On the other hand, if the mutation causes the cell to grow, it is virtually certain that at least one cell in the resulting clone will retain the beneficial, mutated allele when the duplication is lost. That cell will be the progenitor of a stable, "directed" mutant clone. The degree of "direction," however, is only twofold, which is a smaller factor than is observed.

In order for a beneficial mutant to enjoy a stimulation that is 20-fold (for instance) greater than that for a neutral mutant, the number of copies in the amplified array must be 20. There will then be a 1-in-20 chance of preserving an irrelevant mutation when the tandem array is reduced to single copy, while a beneficial mutation will again be preserved with near certainty. Thus, a tandem duplication model *could* work, but the authors have not told us how 20 (or more) tandem copies could accumulate when there is no apparent selective advantage to such a monstrous aberrancy.

The reason that the tandem duplication model does not work well is that there is no property of the moieties of a tandem duplication that allows one copy to be treated differently from any other. However, by introducing the concept of DNA amplification, the model offered does suggest a model that *could* work. (Who knows, maybe CAIRNS and FOSTER had models like this one in mind, too.) Let the required DNA synthesis be "stable DNA replication" (DEMASSY, FAYET and KOGOMA 1984). This DNA synthesis, which appears to be primed here and there by scraps of RNA, is RecA-dependent (WITKIN and KOGOMA 1984). In starving cells, this synthesis will not go far, I suggest, and a full replication fork will not arise from the D-loop. The new chain will be sooner-or-later expelled from the D-loop and degraded. However, if a life-saving mutation arises during the DNA synthesis, it will ensure the wherewithal for turning the D-loop into a full fork, the cell will replicate, and the beneficial mutation will be saved. Voila!

This model (call it "The Toe in the Water Model") can explain most of the data offered in the literature as evidence for the reality of directed mutants. It cannot account, however, for HALL's (1991) claim of strongly correlated reversion of the two mutant sites of a double *trp* mutant. In view of the great difficulty of explaining those revertants by *any* model, it is probably best to reject the observation. In fact, the failure of HALL (1991) to report the results of controls that measure growth of single revertants in colonies of the double *trp* mutant means we needn't take *that* sighting of the unicorn seriously.

How did unicorns get into the act (STAHL 1988, 1990)? In defense of *Nature*'s investigation of analytical procedures in BENVENISTE's laboratory (DAVENAS *et al.* 1988), THE AMAZING RANDI said ". . . what would you do if I said 'I keep a unicorn in my back yard'?" (MADDOX, RANDI and STEWART 1988). RANDI may have been alluding to JAMES THURBER's delightful short story. My allusion was to RANDI's rhetorical question, which invited the answer, "I would climb over the fence to have a look!" I was inviting the readers of *Nature* to have a look at CAIRNS, OVERBAUGH and MILLER (1988), whose claims were counter to conventional wisdom, although not to fundamental scientific laws. The allusion might have been more widely understood if I had retained the final line from the penultimate draft of *A Unicorn in the Garden* (STAHL 1988). That line wondered how far CAIRNS,

OVERBAUGH and MILLER (1988) could dilute the lactose and still see a lactose-directed mutation. By that musing I meant to imply that the authors could expect a reception rather like that received by DAVENAS *et al.* because of the unconventionality of their claims. I did not mean that the work of CAIRNS, OVERBAUGH and MILLER was intrinsically unbelievable.

Some critics (*e.g.*, SMITH 1992) appear to be blindly skeptical of the demonstrations offered in support of the view that cells can mutate in a directed way. By failing to provide a proven (or even attractive) hypothesis, the recent work of FOSTER and CAIRNS (1992) is unlikely to quiet such detractors.

Discussions with JOHN CAIRNS, PAT FOSTER and with members of my laboratory, especially ANDY KUZMINOV, LENA KUZMINOVA, JIM SAWITZKE and RIK MYERS, helped shape this review.

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