

Letter to the Editor

Orders of magnitude change in phenotype rate caused by mutation

To the Editor,

In a forceful article Duesberg and his colleagues [5] reviewed the impressive amount of research results on cancer accumulated over 100 years. It confirms the current conclusion that considering all available experimental results and clinical observations there is no cancer theory without major difficulties, including the prevailing gene-based cancer theories [1,6,13,17]. Phrasing differently, any known cancer theory is refutable to a substantial degree. While the support for their own advocated chromosomal cancer theory appears strong, it seems that their wholesale criticism on mutation cancer theory is premature. In this letter the quantitative evidence against one of their major reasons for dismissing mutation cancer theory, by both *in vivo* experiment and a first principle computation, is explicitly pointed out.

Duesberg et al. [5] listed 9 “absolute discrepancies” or questions which they believe the mutation cancer theory cannot answer:

- “(1) How would non-mutagenic carcinogens cause cancer?”
- (2) What kind of mutation would cause cancer only after delays of several decades and many cell generations?”
- (3) What kind of mutation would alter the phenotype of mutant cells perpetually, despite the absence of further mutagens?”
- (4) What kind of mutation would be able to alter phenotypes at rates that exceed conventional gene mutations 4–11 orders of magnitude?”
- (5) What kind of mutation would generate resistance against many more drugs than the one used to select it?”
- (6) What kind of mutations would change the cellular and nuclear morphologies several-fold within the same “clonal” cancer?”
- (7) What kind of mutation would alter the expressions and metabolic activities of 1000s of genes, which is the hallmark of cancer cells?”

- (8) What kind of mutation would consistently coincide with aneuploidy, although conventional gene mutations generate infinite numbers of new phenotypes without altering the karyotype?”
- (9) Why would cancer not be heritable via conventional mutations by conventional Mendelian genetics?”

Evidently those important questions should be considered seriously by any cancer researcher. There are, however, two general reasons that the temporally inability to address them is not enough to dismiss mutation cancer theory. First, in many situations whether the effects are the causes or the consequences, or mutual causes to each other, are still poorly understood, though their associations with cancers may be obvious. The clarification of such confusing requires further and more experimental and clinical studies. Such efforts have been carrying out, for example, by Weiss et al. [19], Hermsen et al. [7], Weber et al. [18], Bielas et al. [2], Sjoblom et al. [16], and Levitus et al. [8].

Second, the absence of an explanation or of a theory is not a proof that it would not ever exist. If there were already enough amount of consistent experimental and clinical observations, the emergence of a theory would be simply a matter of time. It is a test to our creativity and imagination. Therefore, those “absolute discrepancies” are logically not necessarily against the mutation cancer theory. Having given a “dodged” defense, here I would like to call the attention to one evidence specifically addressing above 4th discrepancy: “(4) What kind of mutation would be able to alter phenotypes at rates that exceed conventional gene mutations 4–11 orders of magnitude?” The question (4) can be answered by mutation cancer theory in a quantitative and first principle manner. The quantitative evidence also suggests answers to questions (1) and (2) of Duesberg et al.

In the phage lambda system, a bio-system arguably started the modern molecular biology [3], extensive and quantitative experiments have demonstrated that simple mutations can cause the change in phenotypes

Table 1

	Phage lambda genotype				
	λ^+ (λO_{R321} , wild type)	$\lambda O_{R3'23'}$	λO_{R121}	λO_{R323}	λO_{R123}
Switching rate (experimental)	2×10^{-9}	5×10^{-7}	3×10^{-6}	2×10^{-5}	∞
Switching rate (calculation)	1×10^{-9}	1×10^{-7}	3×10^{-6}	7×10^{-5}	∞

This table is based on Zhu et al. [20,21]. There are 5 different phage lambda phenotypes, including the “wild type”, which have been systematically studied experimentally [9]. The switching rate is the probability to switch from lysogenic to lytic states per minute under normal lab condition. The symbol ∞ indicates that there is no stable lysogenic state, that is, the phage lambda would immediately switch to lytic state. The switching rate is then “infinite”.

over many orders of magnitude [12,14]. The phenotype easily accessible to experimental study is the switching between lysogenic and lytic states. It is generally known that a mutation in the DNA binding sites can cause more than 100 and more folds change in the switching rate, controlled by a few base pairs in the genomic sequences [4,9,11]. Systematic study showed that at least over 8 orders of magnitude change can be observed among mutants. A quantitative study is summarized in Table 1.

The point should be emphasized is that the mathematical calculation is based on first principle modeling without “free” parameters. What the “first principle” means is that the interaction between involved proteins and the protein–DNA binding are based on carefully reasoned physical, chemical and biological principles during past 40 years. What the “parameter free” means is that all the kinetic parameters needed for the mathematical modeling have been fixed by other experiments. Thus, the remarkable consistency over at least 8 orders of magnitude between the experimental data and mathematical calculation shows that it is unlikely due to artifacts in experiment and/or in modeling. Because such effect can occur in phage lambda, there is no reason that same thing cannot occur in higher organisms [15]: Numerous gene regulatory sites similar to that of phage lambda exist in our human genome and wrong switching in gene regulatory network is generally believed to contribute to cancer. Therefore, the answer to the question (4) of Duesberg et al. based on mutation cancer theory is already positive.

The viability of various mutants, some can live up to thousands of generations before going to lytic state to kill its host *E. coli*, suggests that there can be a long delay in the manifestation in phenotypes after a mutation. Such a gene regulatory example hence directly answers the question (2) of Duesberg et al. In addition,

it is known that the stability of lambda genetic switch can be influenced both chemically and physically, without any mutagenic effect [14,20], that is, non-mutagenic agents can cause the switch from lysogenic to lytic states, therefore changes an otherwise robust phenotype. This fact suggests itself as an answer to the question (1) of Duesberg et al.

To summarize, though whether chromosomal or mutation cancer theory, or both, are the candidates for the cancer theory is too early to call, Duesberg et al. is premature to write out the mutation cancer theory. Even if neither were the final cancer theory, both already appear clinically relevant [10] and should be studied thoroughly.

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