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Commentary

Mismatch repair earns Nobel Prize in Chemistry 2015 to Paul Modrich for a biochemical *tour de force*

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First night after landing from Paris, I was all but sleepless in Seattle when at 3:30 a.m. on October 7, 2015 I was brutally awaken by phone calls from Paris and Zagreb with journalists' question: "What do you have to say about the Nobel Prizes in Chemistry to Lindahl, Sancar and Modrich for DNA repair?". Grudgy, I first answered "Listen, Richard Feynman was mad about being awaken for his own Nobel Prize; why are you awakening me for somebody else's prize!" Jet lagged, I tried to connect to my brain and got back only this: "Nobel in chemistry for DNA repair? Finally. Well merited. Good night. Call me tomorrow". But the professionals made me formulate *ad hoc* compliments for the contributions of my three colleagues and friends. These journalists knew about my long-lasting love affair with DNA repair, in particular mismatch repair (MMR).

DNA repair is the sole fundamental property of DNA that Watson and Crick failed to imagine in 1953. To act in a DNA repair process, the dedicated proteins must recognize chemical abnormalities, the non-DNA entities in DNA, and then either reverse the chemistry to the initial normality (damage reversal) or remove the damaged moiety by excising it out of DNA by molecular surgery and then replacing by insertion of normal nucleotides by correct base-pairing with intact complementary strand (BER and NER). These repair processes were largely biochemically elaborated by Tomas Lindahl and Aziz Sancar. I am honored by the invitation to pay a tribute to Paul Modrich's work on biochemistry of MMR.

There will be two stars in this tribute to Paul Modrich's Nobel Prize: Paul himself and mismatch repair itself. MMR's stardom is based on its being a DNA repair not like others – mechanistically, the most sophisticated repair system that acts as a multifaceted editor of DNA replication, recombination and structure, interfering in all key aspects of genetic stability. To correct DNA copy errors, MMR must make a double diagnostic before acting: (i) diagnostic of chemically normal, but wrong, bases that are mis-matched or non-matched with the complementary strand and (ii) diagnostic of "strandedness", *i.e.*, strand discrimination between the original template strand and the copy strand. Only then does it make sense to remove the mismatched base(s) exclusively from the error-bearing copy strand (otherwise, the original information would also acquire mutation, by its transfer by repair from the erroneous copy strand).

Another reason for MMR stardom is two decades of an exceptional intellectual history created by molecular geneticists while postulating the existence of mismatch correction against the "reason" expressed *e.g.*, by Arthur Kornberg. In the 1974 edition of his book "DNA Replication" A. Kornberg wrote that the fidelity of DNA replication must rely only on DNA polymerases because there is no chemical difference between old and new strand of DNA. I presume that the beautiful (hi)story of MMR is known to few biologists, let alone their students and colleagues. Few would know today that the discovery of MMR required elaboration a special piece of molecular genetics – the genetics of single defined "heteroduplex" DNA molecules reconstituted *in vitro* before being introduced (as single molecules!) into the living cells in order to analyze the progeny of each heteroduplex strand through the successive replication rounds. Such a Hi-Tech sophistication in Lo-Tech times was developed in Matthew Meselson's and Maurice Fox's laboratories [1–3]. I wish to walk you through the tale of MMR in the period preceding Paul Modrich's research that finally revealed its molecular intricacies. To avoid redundancy with many comprehensive scholarly reviews on MMR (*e.g.*, [4–8]), I will write about MMR mainly by describing ideas, people and some experiments, but in particular about the biological ramifications of MMR that are almost absent in the literature. Paul's brilliant breakthrough research on MMR biochemistry that earned him the Nobel Prize in Chemistry was well reviewed [4–8]. I wish to share a fascinating evolution of concepts.

1. The ideas

My medical students know for sure that MMR was discovered as the genetic defect in the hereditary non-polyposis colon cancer (HNPCC) syndrome "way back in 1993". Yet, the concept of MMR arose around 1964 (almost simultaneously, and probably independently, by Harriett Ephrusi-Taylor, Robin Holliday and Matthew Meselson) to explain aberrant segregation (called gene conversion) of mutations used as genetic markers in genetic crosses with fungi and yeast [9]. Gene conversion looks as if allelic mutations were hopping from one homologous DNA molecule to another during the act of genetic recombination – a sort of genetic infection involving the same sites in DNA molecules! Strand exchange was invoked to form the heteroduplex "splice" or "patch" region in which one

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partner strand carried mutation (genetic marker) and the other not – hence the formation of a mismatched base-pair. The geneticists hypothesized that "mismatch repair" could either remove the mutation from the invading strand (and they would be blind to this event) or fix it, depending which strand was repaired by MMR. Nobody knew what would be the benefit of such DNA repair.

The idea of a benefit for acquisition of MMR came from the observed coincidence of two phenotypes of MMR deficiency in bacteria: (i) the loss of "marker effect" – a version of gene conversion in bacterial genetic recombination [8] and (ii) the "mutator" phenotype displaying high spontaneous mutation rates. The coincidence and inseparability of the two phenotypes was noticed first in Gerard Thiraby's Ph.D. thesis (University of Toulouse, 1969) study of the transformation of *Streptococcus pneumoniae hex* mutant [8]. Gerard was, *via* his thesis adviser Michel Sicard, a second-generation student of Ephrussi-Taylor, who, to account for "marker effects" in pneumococcal transformation, postulated the existence of mismatch repair in 1964/1965 (reviewed in 8). Marker effect is sort of uncertainty principle in genetic recombination: recombination frequency between closely and equally spaced markers (*i.e.*, mutations) can vary several orders of magnitude depending solely on the chemical nature of mutation [10]. This was interpreted as the consequence of variable patterns and efficacies of MMR in the DNA heteroduplex region. When genetic markers from both crossing partners are closely spaced, they tend to be included in the same heteroduplex joint region and form two mismatched base-pairs (when two genetic markers are used) that can be repaired either on the same strand (*i.e.*, co-repaired), or on opposite strands (repaired in trans), or remain unrepaired. When the genetic markers are associated with distinct phenotypes, the geneticist will observe different genetic outcomes and identify each of the three cases. This interpretation was abstract (try a simple drawing) and hypothetical, derived from results of tooth-picking colonies on solid agar media.

According to Maury Fox (pers. comm.), the pneumococcal *hex* mutator mutant emerged spontaneously in the Rockefeller Institute in the 1940s, possibly because of its accelerated (mutational) adaptation to the early less-than-optimal growth media, or was sorted out for its efficient transformation even with the low efficiency transformation markers (in normal bacteria). The "loss of marker effect" phenotype in *hex* (initially observed by Sanford Lacks; see ref. [8]) means that all markers/mutations are high efficiency markers because all remain unrepaired when transferred and integrated in DNA heteroduplex, therefore remain stably incorporated in the chromosome and transmitted to the progeny.

2. Prelude to the discovery of post-replication error correction in DNA

The implications of the *hex* mutator phenotype were discussed in depth during the 1975 EMBO Recombination Meeting in the Scottish town of Nethybridge. The obvious question was: to correct DNA copy errors, how can MMR proteins "know" which DNA strand is the original and which is the copy? The simplest way is to imagine that MMR complex somehow hangs on the new strand, *e.g.*, by hooking onto, and staying behind, the DNA polymerase complex on the nascent strand. But, we knew that, to be detected, MMR must and does occur on artificially constructed DNA heteroduplex before its replication [1–3]. Robin Holliday's proposition that the discontinuities in the newly synthesized strands could be the signals for strand discrimination was not met with enthusiasm. The reason was that the standard model of DNA replication involves "leading" and "lagging" strand synthesis and only the lagging strand would be subject to such mismatch correction reducing mutations only by 50%. But now, it turns out that R. Holliday's proposition probably applies to the vast majority of bacteria and to all other organisms. The forgotten fact is that *Escherichia coli* and yeast mutants with a temperature sensitive DNA ligase synthesize, at the elevated temperature, all its DNA discontinuously and of the same size (Okazaki fragments), suggesting that both "lagging" and "leading" strands might be initially synthesized in a discontinuous fashion [11,12]. Perhaps the strand-biased ligation kinetics could produce the leading/lagging strand asymmetry? Clearly, a selective mismatch-stimulated abortion of error-bearing Okazaki fragments by a helicase would result in effective copy error correction.

An alternative idea occurred to Matt Meselson who proposed that the methylation status of DNA strands could direct mismatch repair to the copy strand [2]. While sipping malt whisky in the Nethybridge hotel, he told me in 1975: "If I were the mismatch repair enzyme, I would look for DNA methylation pattern". Matt knew from his *E. coli* restriction/modification studies that DNA strand modification – *via* sequence-specific addition of methyl groups – lags after the strand synthesis leaving a limited time window when newly synthesized strands are transiently undermethylated. That window could be used by mismatch repair enzymes to discriminate strands and repair only the transiently undermethylated copy strand! Few days before the trip to Scotland, I read a paper by M. G. Marinus and N.G. Morris about isolation of *E. coli* mutants deficient in either adenine (*dam*) or cytosine (*dcm*) methylation [13]. They noticed that *dam*, but not *dcm*, mutants exhibited increased spontaneous mutation rates – a mutator phenotype! Thus, the Dam-methylation (6meAdo in GATC sequences) fulfilled the prediction of Meselson's hypothesis because, in non-methylated DNA of the *dam* mutant, a random undirected mismatch repair should cause a mutator effect as severe as the mismatch repair deficiency itself. I was excited by the amazing coincidence, but Matt was typically skeptical even about his own idea: too good to be true!

I had a personal reason to be excited about a likely clue to the mismatch repair discovery because in the period 1970–1973 I ran in Matt Meselson's lab a few thousand of alkaline sucrose velocity gradients of radioactive λ phage DNA (reconstituted *in vitro* as to bear 3 equidistant mismatches) looking for *E. coli* cell extract activity that would cut DNA strand at mismatches and produce (multiples of) 1/4 strand length fragments. Unfortunately for me, there was often a very weak signal that would vanish by purification and I never succeeded in isolating the elusive MMR endonuclease. The reason for my failure is that Matt's later intuition about DNA methylation in MMR was correct for *E. coli* and that my λ phage DNA preparations were about 70% methylated at GATC sequences. The bottleneck to repair activity was in the substrate. During my desperate years Matt used to leave short messages on my desk like "virtue will triumph!". It eventually did 3 years later for another kind of experiment, but even more for Paul Modrich a decade later.

3. First direct evidence for methyl-directed mismatch repair

On May 28, 1976, during my one-month visit to test the DNA methylation hypothesis, Mathew Meselson, Robert Wagner and I saw the first direct experimental evidence of the methyl-directed mismatch repair process in *E. coli*. To imitate copy errors in the newly synthesized, transiently under-methylated, DNA strands, I used *dam* mutants to produce unmethylated λ DNA and with Bob Wagner made the first use of a hemi-methylated (one strand methylated, the other not) DNA heteroduplex with single mismatched base pairs as genetic markers. The result appeared exactly as imagined by Matt Meselson, *i.e.*, as if MMR were designed to correct copy errors in DNA replication. Mismatched markers were lost from the transfected λ DNA heteroduplex only in the