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MILESTONE 9

Transformers, elements in disguise

In the post-genomic era, understanding biological processes increasingly relies on analysis of gene functions. Yet, until a few decades ago, studying eukaryotic gene function *in vivo* was impossible, as no efficient and reproducible procedures to transfer DNA into eukaryotic cells were available.

In the late 1970s, Gerald Fink and colleagues set the basis for studying eukaryotic genes by establishing a transformation protocol to introduce exogenous DNA into yeast cells permanently. A few years later, Mario Capecchi showed that microinjection of the herpes simplex virus gene encoding thymidine kinase into the nuclei of mammalian cells lacking this enzyme allowed the kinase activity to be recovered.

It was only in 1982, however, that DNA was successfully manipulated *in vivo* in a higher organism. Allan Spradling and Gerald Rubin characterized P-elements — mobile DNA elements — through analysis of *Drosophila melanogaster* strains that gave rise to progeny suffering from the hybrid dysgenesis genetic syndrome. They identified two groups of P-elements that differed in size and ability to move within the genome. Injecting 3-kb autonomous P-elements into *Drosophila* embryos lacking them revealed that the elements could insert into random genomic positions, inducing mutations in a fraction of the progeny. These findings initiated the use of P-elements in large-scale mutagenesis screens in *Drosophila*, given the advantage of gene cloning in the identified mutants.

In mice, site-directed mutagenesis was first used in 1987, when two research teams targeted the gene encoding hypoxanthine phosphoribosyl transferase (*Hprt*) by homologous recombination in embryonic stem (ES) cells. These cells were chosen for their unique potential to be manipulated *in vitro* and reintroduced into mouse blastocysts, producing chimeric animals that can transmit the new traits to subsequent generations. Kirk Thomas and Mario Capecchi engineered two classes of vectors that efficiently disrupted *Hprt* either by replacing endogenous sequences with the exogenous neomycin-resistance gene or by inserting the exogenous sequence into the *Hprt* locus. They then identified ES cells carrying mutated genes by selecting for acquired resistance to the drug G418 and the base analogue 6-TG. Oliver Smithies and co-workers also used this technique to correct the defects of three independent *Hprt*-mutated ES cell lines. Together, these ground-breaking studies paved the way for functional genomics and gene therapy.

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