1. **What is the significance of this research topic?**

Restriction enzymes (REase) protect bacteria and archaea from infection by viruses DNA molecules. In 1952-1953, Luria and Human observe that bacteriophages have a different ability to grow on different host strains. Once established on one strain, the phages would grow easily on this strain but were “restricted” initially to grow on new strains. Arber and Dussoix postulated, that certain bacterial strains contain an endonuclease that is able to cleave DNA and that, some strains have a strain modification protecting them from their own endonuclease.

Foreign DNA, such as the one from an infecting phage, is degraded by the endonuclease, restricting its ability to infect its host (hence the term “restriction endonuclease”). In some cases, a small portion of the phage DNA, is modified prior to degradation by the endonuclease. This modified DNA is able to successfully replicate, and infect other hosts, but since the new host does not contain the protecting system modification as the first one, the modified phage cannot replicate in the new host.

* **Class 1**, REase *EcoKI*, was the first to be purified. Enzymes of type I, are very large (with a molecular weight of about 300-400 kDa). They consist in 5 subunits of 3 different proteins, and require the cofactors Mg2+, ATP and S-adenosyl methionine (SAM) for activity. Because these enzymes cuts DNA randomly far from their recognition sequence, they cannot be used as cloning reagents.
* A breakthrough came after two seminal papers from Smith’s laboratory, Smith and al. described the properties of *Haemophilus influenzae* endonuclease, *HindII*, which, unlike *EcoKI*, requires only Mg2+ for activity. *HindII*, like any **type II enzyme**, cleaves the two strands of DNA at a fixed location, for *HindII*, within its recognition sequence (GTY|RAC) (Y=pyrimidine (C or T), R=purine (A or G)), into two fragments with “blunt” ends. An important feature of the cut, is its palindromic nature, a nucleotide sequence on one strand segment is now complementary of segment on the other strand. Type II, enzymes are smaller (molecular weight about 100-20kDa), and have identical subunits. Nathan’s laboratory further investigated Smith work, and discovered that individual gene could be mapped by comparing the endonuclease “restriction maps” with the corresponding genetic map.
* **Type III and IV** enzymes are rare. Type III enzyme is a large molecule (mol. weight ~ 200KDa), they do require Mg2+ and ATP for activation but not SAM. They cleave outside of their recognition sequences and require these two DNA sequences in opposite directions and rarely give complete digests. Type IV recognize methylated DNA.
* There are today more than 3,600 enzymes representing 250 different specificities (latest total number can be found at http://rebase.neb.com/rebase/rebase.html).

Restriction enzymes (REase) have been the workhorse of molecular biology for cloning, generation of libraries, DNA sequencing, production of hormones and played a critical role in recombinant DNA technology (**rDNA**): when Boyer and Cohen used DNA ligase to join a DNA molecule with “sticky” ends produced by cleavage with EcoRI restriction endonuclease, to a plasmid DNA molecule also cleaved by EcoRI, they launched the era of recombinant DNA (rDNA). Numbers of discoveries have stemmed from gene cloning and rDNA. Enzymes themselves have been improved by rDNA. Today every commercial enzyme has been purified from *an E. coli.* Small quantities of genes are isolated from the desired proteins, moved to *E. coli*, transcribed, translated again into proteins at optimal rates, high yield and high levels of purity by avoiding contaminating factors.

The restriction enzymes have transformed biology and medicine, increasing greatly understanding of a variety of forms of life. They also enabled the biotechnology revolution.

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1. **Who is working in this area?**
2. **What methods are used to study the concepts described in the paper?**
3. **Does the review article lead to new questions or hypotheses in this technical area (by the authors, by other researchers)?**
4. **What are some practical applications of the research discussed in the article?**

One of the first application of REases, was their use as molecular weight markers for gel electrophoresis; for example, the restriction enzyme *HindIII* digestion of *E. coli* produced 8 restriction fragments with precise molecular weights. Restriction enzymes also allowed the detection of f homologous DNA sequences, or polymorphisms. Restriction length polymorphism (RFLP) analysis which has been which superseded by faster and cheaper techniques, was initially used in genome mapping, localization of genes for genetic diseases, and genetic fingerprinting. Today terminal restriction fragment length polymorphism (TRFLP) is used for characterizing bacterial , fungi species or microbial communities.

**6. How does this topic relate to other areas of cell biology, bioengineering, or medicine?**