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 but also 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 phage is able to successfully replicate, and infect other hosts, but since the new host does not contain the protecting system modification as the first initial host, 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 Mg^{2+}, ATP and S-adenosyl methionine (SAM) for activity. Because these enzymes cuts DNA randomly far from their recognition sequence, they cannot be easily used as cloning reagents.
- In 1970's, 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 Mg^{2+} for activity. *HindII*, like any **type II enzyme**, cleaves the two strands of DNA at a fixed location into two fragments with "blunt" ends: for *HindII* within its recognition sequence (GTY|RAC) (Y=pyrimidine (C or T), R=purine (A or G)). 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. Pieces of DNA cut with the same restriction enzymes would therefore all have the same length and could be joined to create hybrid DNA molecules. Type II enzymes are smaller (molecular weight about 100-20kDa) and have identical subunits.
- **Type III and IV** enzymes are rare. Type III enzyme is a large molecule (mol. weight ~ 200KDa), they do require Mg^{2+} and ATP for activation but not SAM. They cleave outside of their recognition sequences and require two DNA sequences within the same DNA in opposite directions, and rarely give complete digests. Type IV enzymes recognize methylated DNA.
- These days there are more than 3,600 enzymes representing more than 250 different specificities (latest numbers can be found at http://rebase.neb.com/rebase/rebase.html).

Restriction enzymes (REase) 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. 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 significantly the understanding of many forms of life.

2. Who is working in this area?

The companies like NEB (New England Biolabs) or Promega are still in activity today, they were among the first companies to discover, purify, produce restriction enzymes. With time they have accumulated an impressive catalog of restriction endonucleases but also PCR amplification products, nucleic acid purification products, RNA reagents, DNA assembly, cloning and mutagenesis kits and genome editing tools.
Moore recent new comers are: Life in Genomics, Arbor Sciences, Watchmaker Genomics and Eton Science. Other companies were founded to exploit REases to produce high value molecule based therapeutics like Biogen which engineered the first "recombinant" vaccine for hepatitis B or Genentech which was able to create human insulin in *E.coli*.
In academia, restriction enzymes are ubiquitous in any biological or life science research. Recently Fordyce and Hersschlag labs created a high-throughput microfluidic kinetics (HT-MEK) chip that dramatically speeds up enzyme analysis. HT-MEK could accelerate allosteric targeting drugs, and allows to reverse-engineer enzymes and design for example enzymes that can degrade plastics into nontoxic pieces.

3. What methods are used to study the concepts described in the paper?

4. Does the review article lead to new questions or hypotheses in this technical area (by the authors, by other researchers)?

5. 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* produces 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 superseded by faster and cheaper techniques, was initially used in genome mapping, localization of genes for genetic diseases, and genetic fingerprinting. Today single-nucleotides polymorphism (SNP) analysis is preferred, and sequence changes can also be analyzed faster with polymerase chain reaction (PCR). Terminal restriction fragment length polymorphism (TRFLP) detection, characterizes bacterial, fungi species or microbial communities; PCR products are digested using restriction enzymes. Restriction enzymes, *Mbo I and Hpa II*, played a critical role in the identification of CpG islands which are DNA methylation regions crucial for gene expression.

Restriction enzymes (REase) have been the workhorse of molecular biology for:

Cloning: initially Stanley Cohen and his colleagues incorporated foreign DNA into natural plasmids to create cloning-plasmid vectors that self-propagate in *E. coli.* Restriction enzymes have been useful tools in PCR for confirming that insertions have taken place successfuly.
DNA mapping:
In the 1970s, Nathans mapped the functional units of the SV40 genome, by discovering that individual gene could be mapped onto the endonuclease "restriction maps". They also found that genetic mutations could be detected using restriction endonuclease digestion. This work evolved into SNP and insertion/deletions (Indels) detection.
Markers of epigenetic modifications: Restriction Landmark Genome Scanning (RLGS) is a gel electrophoresis mapping techniques in which *NotI*, *AscI*, *EagI* or *BssHII* enzymes identify changes in the methylation patterns of the human genome during the development of cancer cells.
Synthetic biology: aims to redesign, or create biological systems to enhance existing or create new abilities. Some DNA assembly technologies are based on REase-ligation methodology, and REases abilities to cleave DNA outside of the recognition sequence (exonucleases activity).
DNA libraries: the use of type IIS enzymes as tagging enzymes, have allowed the identification and quantification of a large numbers of mRNA transcripts. For example, REases (like *AcuI* and *USER*) have been used to insert tags into sample DNA, which is amplified to form long, single-stranded DNA "nanoballs" that are templates in chip-based sequencing-by-litigation methodology.
Nicking enzymes: Some type IIS enzymes have two different catalytic sites. By inactivating one catalytic site at a time, these enzymes are converted strand-specific DNA nicking enzymes (cleaving only one DNA strand) and are useful molecular investigating tools for research on altering DNA, generation of reporter plasmids with modified structures, and creation of a DNA motor to transport DNA payload.
In vivo gene editing: to correct mutations that causes genetic disease (or any other ethical genetic mutation): site-specific-cleavages were realized using Zinc Finger Nucleases (ZFNs) and Transcription-Activator-like Effector Nucleases (TALENs). These techniques have since been replaced by the more precise CRISPR-Cas enzyme system, which can cut DNA within a long target sequence (~30 bp) and can be more easily reprogrammed for new targets. The CRISPR-Cas uses a small piece of RNA to recognize the intended DNA sequence, and guide the Cas 9 enzyme to cut the DNA at the targeted sequence.

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