# Pulse Chase Experiment

## Tracking molecular Movement

- **Pulse-chase experiment is a commonly used technique in molecular biology to track the movement of molecules, such as proteins or nucleic acids, within a cell or organism**
- **The experiment involves labeling a precursor molecule, introducing it into the system, replacing it with an unlabeled one, and tracking the labeled molecule over time**
- **The technique has numerous applications in molecular biology and can provide insights into cellular processes such as protein synthesis, degradation, and trafficking**

# Steps involved in a typical pulse-chase experiment

## 1.Labeling the Precursor Molecule:

- The first step in a pulse-chase experiment is to label the precursor molecule with a detectable marker
- This can be achieved using a variety of methods such as radioactive labeling, fluorescent labeling, or biotinylation
- For example, if the experiment is designed to track protein movement, a radioactive amino acid such as ^35S-methionine can be used to label the newly synthesized proteins

## 2. Pulse:

- In the next step, the labeled precursor molecule is introduced into the system, usually by adding it to the culture medium of cells or administering it to an organism
- The labeled molecule is allowed to incorporate into the target molecules such as proteins or nucleic acids.

## 3. Chase:

- Once the labeled precursor has been introduced into the system, the chase step is initiated
- This involves removing the labeled precursor molecule and replacing it with an unlabeled one
- This allows for the detection of the labeled molecule over time as it moves through the system

## 4.Collection of Samples:

- After the chase period, samples are collected at different time points to determine the location and fate of the labeled molecules
- The samples can be collected from cells or tissues, or from different organs of an organism

## 5.Detection and Analysis of Labeled Molecules:

- The final step involves the detection and analysis of the labeled molecules in the collected samples
- This can be achieved using a variety of methods such as autoradiography, fluorescence microscopy, or western blotting
- By comparing the signal intensity of the labeled molecule at different time points, it is possible to determine the rate of movement or turnover of the labeled molecule within the system

# Applications

## 1. Analysis of the in vivo assembly of bacteriophage T4 tail

- Bacteriophage T4 mutants defective in the head assembly were used to infect the E. coli cultures to study tail assembly in isolation
- The bacterial cultures were labeled with [3H] leucine on the onset of late protein synthesis.
- After the complete tail began to accumulate at a constant rate, the bacterial cultures were pulsed with [35S] methionine, and then chased
- Completed tails were obtained and purified at one-minute intervals for the next 30 minutes
- It was found that the closer the assembly point to the end of the pathway, the sooner the chase appears, presenting the assembly cascade

## 2. Assessment of MHC class II biosynthesis, maturation, and peptide loading

- To study the major histocompatibility complex (MHC) class II synthesis, maturation, trafficking, and peptide loading in human Epstein-Barr virus-transformed B-lymphoblastoid cell lines (B-LCL)
- The MHC class II glycoproteins bind heterogeneous mixtures of peptides presented on the surface of antigen-presenting cells for the inspection of CD4+ T helper lymphocytes
- The results obtained from these experiments can illustrate information to track changes in the molecular associations, an abundance of radiolabeled proteins, and the conformation-sensitive monoclonal antibodies (mAbs)

## 3. Biosynthesis, targeting, and processing of lysosomal proteins

- The radioactive amino acids and modifier groups are incorporated into proteins to study their life cycle and various modifications
- Lysosomal enzymes can also be detected and characterized using pulse-chase analysis
- The pulse-chase labeling provides a detailed insight into organelle-specific modifications of lysosomal proteins
- For this, an antibody against lysosomal protease is used as a reference
- The pulse-chase experiment demonstrates the conversion of the precursor into the mature form

## 4. Assessment of protein maturation and degradation

- Using the short pulses, the whole process of protein synthesis to degradation can be determined in a natural environment
- The technique has been widely used for endogenous and viral proteins including thyroglobulin, vesicular stomatitis virus, influenza hemagglutinin, envelope proteins, and immunoglobulins
- This biochemical approach is also used for study of protein folding, post-translational modifications, endogenous and intracellular transport, and the rate of protein degradation

# Strengths and Limitations

- The pulse-chase analysis is a powerful technique used for the assessment of protein synthesis, trafficking, and degradation
- The technique could also be used to monitor the binding of proteins and the kinetics of the processes
- The pulse-chase method is widely used to monitor the assembly cascade of viruses
- The method should be readily applicable to the detection and assessment of multicomponent regulatory complexes and macromolecular machines accessible to the biochemical and biophysical investigation
- The approach is limited to monitoring the kinetics of stable interactions
- The labeling of the proteins could be challenging for low-abundant proteins