

Abstract

A large number of studies published in the last years shows how a population of cells is characterised by a high cell-to-cell variability. Even by considering monoclonal and identically prepared single bacteria, these give rise to highly diverse dynamics: some of them may be able to form colonies, while others do not. Such strong heterogeneity finds its roots in the stochasticity of gene expression, that leads single cell to express given genes into proteins at given levels at a certain time. However, classical experimental techniques typically analyse large population of cells and are not suitable to highlight heterogeneity at the individual level. It is only in the last decade, thanks to the development of innovative experimental techniques (e.g. single cell sequencing, FACS, etc.), that we can quantify and thus model this heterogeneity. Thus, such effects call the development of stochastic models able to both describe and make predictions of the system at a macroscopic scale by taking into account the microscopic diversity.

In this thesis we aim at investigating such heterogeneity of a population of cells under different points of view. On one side we analyse how cell-to-cell variability influences the growth of the entire population, by studying the main aspects that characterise the macroscopic growth. On the other side, we focus on a molecular level and investigate a mechanism of gene regulation that may lead to bimodal phenotypes. In both cases, we adopt an approach of investigation that can be summarised in the following loop: we begin by developing simple minimal mathematical models, then we experimentally test its prediction through quantitative, systematic measurements of variables representative of the system, and finally we modify the initial model hypothesis in light of the empirical results.

The dynamics of growth of a population of cells is the topic of the first part of this thesis. Here, we investigate the dependence on the initial conditions of different phases of growth firstly by developing two mathematical models, one for

each interesting growth phase (i.e. adaptive phase and exponential growth phase). By defining a robust experimental protocol for cell growth, we then test the predictions of the models on a widely studied cancer cell line (Jurkat). Following this approach, we suppose a possible mechanism of communication among cells on which the entire population dynamics may rely. We then investigate a second aspect of cell growth, namely its relation with gene expression. Recent works performed on growing bacterial colonies, by starting from simple empirical relations between the physiological state of a population and its gene expression, have developed an entire theory of bacterial growth. This is a model that assumes proteome partitioning and is able to be predictive even in absence of a complete knowledge of molecular details. Indeed, it has been used for predicting a wide spectrum of bacterial behaviours that range from antibiotic resistance to unnecessary protein production. Given the lack of similar studies for mammalian cells, and based on a large range of analogies between bacteria and cancer cells, we transfer the same approach to cancer cells.

The second part of the thesis is devoted to the study of the heterogeneity of a population of cells under a molecular point of view. In particular, we focus on the role of cell-to-cell variability on a peculiar mechanism of post-transcriptional gene regulation mediated by microRNAs (miRNAs). miRNAs are small non coding RNA molecules able to bind to other messenger RNAs (mRNAs) and downregulate their expression. It has been found that such regulation may lead the system to bimodal distributions in the expression of the target mRNA, usually fingerprint of the presence of two distinct phenotypes. Moreover, the nature of the interaction between miRNAs and their targets gives rise to a complex network of miRNAs interacting with several mRNA targets. Such targets may then cross-regulate each other in an indirect miRNA-mediated manner. This effect, called “competing endogenous RNA (ceRNA) effect”, has remarkable properties even in presence of extrinsic noise, i.e. fluctuations that affect all the components of the system. While miRNA-mediated interactions have been widely characterised from a theoretical point of view in the past years, quantitative experiments on the ceRNA effects are much more recent. Here, we first review the stochastic models developed to describe the miRNA-mediated gene expression, pointing out the predictions that may be experimentally investigated. Second, we present the experimental setup we used to test such predictions. Last, we focus on the experiments performed to test the influence of extrinsic noise on miRNA-target interaction.