# THE INITIATION OF TRANSCRIPTION AT 54-DEPENDENT PROMOTERS

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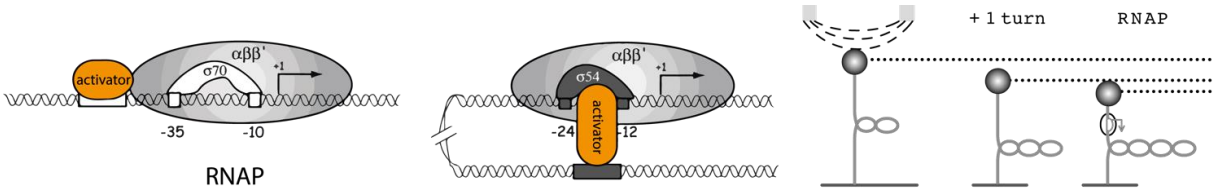
Transcription initiation (TI) by RNA polymerase (RNAP) is a highly dynamic and regulated process. Central to this process are activators that stimulate DNA unwinding by the bacterial RNAP holoenzyme (RNAP and its sigma factor). For σ70-dependent promoters (regulating house-keeping genes in bacteria), activators are dispensable. The situation is different for σ54-dependent promoters (regulating stress-response and virulence genes). There, activators are ATPases that remodel their substrate to exert their function. To date, the exact molecular mechanisms involved in the TI by σ54 remain largely unknown. Using purified components, we will perform **single-molecule experiments** (using a state-of-the art Magnetic Tweezers that we have developed) and **identify regulatory hotspots** in σ54-dependent TI (with potential applications for molecular therapeutics). In addition, we will investigate a new class of activators (not yet reported), which could activate both σ54- and σ70-dependent promoters.

Knowledge of biology is not required but the successful candidate should show a strong interest for molecular biology and biochemistry (part of the biological samples will be obtained in collaboration).




*Left. The two routes of bacterial transcription initiation and the role of activators (RNA Polymerase, composed of five conserved sub-units). The mode of activation of these two types of transcription is drastically different and so activators are specific to either types of transcription. Right. Magnetic Tweezers. A dsDNA (around 3,000 bp) is attached between a glass surface and a magnetic bead (1 micron in diameter). If the dsDNA molecule is devoid of nicks and the attachments at both ends are rotationally constrained, a full rotation of the magnetic bead changes the number of plectonemes (the macroscopic loops) by one unit. Similarly, at a given rotation, a change in twist of the helix induces a change in the number of plectonemes (and consequently a change in the extension). As RNA Polymerase (en route for transcription) unwinds DNA at the promoter sequence, its activity (or the change in the bead's height) can be followed in real time.* Therefore **MT** (which is sensitive to a change in transcription bubble size of one bp) **is a unique tool to study transcription**.