The 3-dimensional transcription film

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Gene expression takes place in two stages: the transcription of DNA to RNA by an enzyme called RNA polymerase, followed by the translation of this RNA into proteins, whose behaviour affects the characteristics of each individual.

Transcription involves about fifty regulatory molecules that interact with each other to begin reading the gene at the right place and the right time. The slightest irregularity of one of these molecules disturbs the transcription. An understanding of the initiation and regulation mechanisms is essential in order to understand gene expression.

The structural biology researchers at IGBMC, France, are studying molecular structures to gain a better understanding of how they function. Patrick Schultz's team is particularly focusing on the architecture of the molecules involved in transcription and attempting to decode the mechanisms of their interactions.

An analysis of the transcription complexes by electron cryomicroscopy allows a molecule to be observed in a hydrated state close to its natural state. Each photograph, taken using a microscope, shows thousands of specimens of the same molecule from different angles and at different instants in their reaction cycle. The statistical analysis of these images performed by Patrick Schultz's team revealed different conformations in three dimensions, which correspond to different stages of transcription initiation. 'We performed image-by-image sequencing and made a film of the initial stages of transcription,' says Schultz.

Patrick Schultz's team is interested in a complex protein that acts as an assembly platform in the initiation phase of transcription: the factor TFIID. Through interaction with the activator Rap1, bound upstream from the gene to be transcribed, it is attracted to the DNA and binds onto it. Combined with another factor, TFIIA, it changes conformation and allows the RNA polymerase to initiate transcription. The original aspect of this mechanism is based on the formation of a DNA loop, which allows the RNA polymerase to be positioned exactly at the start of the sequence of the gene to be transcribed.

The structure of the transcription factor TFIID obtained after image analysis is represented in yellow on an electron cryomicroscopy image background, showing the frozen hydrated molecules in dark grey. The transcription activator Rap1 (red) interacts with the factor TFIIA (blue) and contributes to forming a DNA loop (green).

The biological molecules in living organisms exist in an aqueous environment, which must be preserved whilst observing the molecules. In order to be 'seen', however, molecules must be placed in an electron microscope, which operates in a vacuum and dehydrates the sample. The solution, developed in the 1980s, is to use refrigeration to keep the specimen hydrated and to examine it by electron cryomicroscopy. A very thin film (approximately 100 nm, or one ten-thousandth of a millimetre thick) of the suspension containing the sample to be analysed must be created in order to be transparent to electrons. This film is cooled very rapidly (at a rate of approximately 10,000°C per second) by plunging it into liquid ethane cooled to -170°C. This freezing speed prevents the formation of ice crystals, and the sample is trapped in a layer of vitrified water. The cold chain must be maintained throughout the observation period using a cold plate. The molecules are hydrated and observed without contrast agent.

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