DBIRD, a driving force behind protein diversity

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Recently discovered and still poorly described, alternative splicing plays a predominant role in protein diversity of organisms. Researchers from Liège, London and Munich have uncovered a new complex protein, DBIRD, which is involved in the regulation of this process. The results of their work have been published in the journal *Nature*.

Many of us have heard of "splicing" in school or at university. Indeed, this process which has been known for a long time is an integral part of the chapter relating to the transcription of DNA into messenger RNA. As a reminder, splicing occurs when polymerase RNA II has created pre-messenger RNA from single strand DNA corresponding to the gene to be expressed. This pre-messenger RNA is a mosaic of coding sequences, the exons, and non-coding sequences, the introns. In order to obtain a pure messenger RNA ready to be translated into protein, the pre-messenger RNA must be cleansed of the non-coding sequences that it is composed of. In this way the introns are eliminated and the exons are interlinked to form mature messenger RNA, which is called the splicing process.
Until quite recently, students were taught that a gene codes for a protein by the intermediary of mature messenger RNA stripped of introns. However, things have changed in the last few years! This is not to say that cells have suddenly changed their way of working but scientists have discovered a mechanism that remained hidden up to this point. It is called alternative splicing. 

"Alternative splicing does not use the factors involved in normal splicing", explains Pierre Close, a postdoctoral researcher at the F.R.S.-FNRS in the medical chemistry unit of GIGA directed by Alain Chariot. "It is a mechanism that is still relatively unknown because most of studies concerning this process have only been published over the last two or three years", he continues. One thing is certain, however, alternative splicing increases protein diversity. In fact when a gene, or rather the pre-messenger RNA emanating from this gene, is subject to alternative splicing, it can, depending on the circumstances, give rise to different mature messenger RNAs and therefore different proteins. Why? Quite simply because alternative splicing does not necessarily cause exclusion of all the introns and inclusion of all the exons in the final RNA molecule. According to poorly understood events one or other of the introns can
be kept in the mature messenger RNA and one or other of the exons can be excluded. This is how different proteins are produced, or more precisely, **protein isoforms**.

**A biochemical challenge**

According to recent studies, 95-100% of human pre-mRNAs are subject to regulation by alternative splicing. "The consequences of this type of splicing are still rarely studied, several scientific publications have recently been released and they underline the role of alternative splicing in oncogene regulation. Some oncogenes are indeed more powerful according to the way they are spliced", indicates the researcher Pierre Close. Gene regulation by alternative splicing has up to the present mainly been studied in relation to cancers and neurodegenerative diseases.

Thanks to a grant from EMBO (European Molecular Biology Organization), Pierre Close joined the laboratory of Dr Jesper Svejstrup at Cancer Research UK, **London Research Institute**, Clare Hall Laboratories, for a post-doctoral internship. "The laboratory of Dr Svejstrup studies the biochemical mechanisms of **gene transcription** in the wider sense. That was the starting point of the present study. The objective of the project was to establish new connections between newly -synthesized RNA and transcription by RNA polymerase II by purifying complexes associated with nascent RNA". In order to do this, the researchers rose to a huge technical challenge in biochemical terms: "We purified protein complexes associated with nascent RNA and therefore still connected to RNA polymerase II, at the chromatin level", explains Pierre Close.

Among the purified complex proteins, the scientists identified one which was particularly interesting because it was linked to the formation of RNA and to transcription. "We named it DBIRD. This protein complex is composed of two proteins: DBC1 (Deleted in Breast Cancer protein 1) and another protein that was hitherto unknown, which we called ZIRD (ZNF-protein interacting with nuclear RNPs and DBC1)", continues Pierre Close.

**DBIRD speeds things up**

Once DBIRD was identified and named, the scientists applied themselves to describing the role that this protein complex could play in transcription. "We carried out studies of **DNA-microarray** (or DNA chips) which allowed us to measure the abundance of each exon in the cells at a given moment", explains Pierre Close. "We used control cells and deficient cells for the DBIRD complex and we analyzed the abundance of each exon in the two cell- types", he continues. In this way the researchers obtained a list of about 3000 exons whose abundance varied according to the presence or the absence of DBIRD in the cell. "These exons are therefore spliced differently according to whether DBIRD is functional or not", specifies Pierre Close.

To understand how DBIRD influences the splicing of these exons, the scientists carried out a bioinformatics study of these sequences in collaboration with a team from the **Munich Gene Center** (Germany). "We found a quite powerful correlation between the abundance of pairs of nucleic acid bases adenine-thymine (A/T) and the exons which were differently spliced", the researcher reveals. Thus the sequences rich in A/T sequences would be particularly sensitive to the DBIRD complex because they are more or less included in the final messenger RNA according to the absence or presence of this complex.

It remains to be understood how DBIRD regulates the inclusion or exclusion of these exons. "In a normal cell, the RNA polymerase II moves along the single strand DNA corresponding to the gene and transcribes it. When this enzyme complex meets an exon rich in A/T this poses a problem because these are sequences that are difficult to transcribe", explains Pierre Close. "We think that the DBIRD complex somehow helps the RNA polymerase II to transcribe these sequences rich in A/T, by a mechanism we have yet to understand", © Université de Liège - http://reflexions.ulg.ac.be/ - 20 May 2017 - 3 -
continues the post-doctoral researcher. This is what the results of the measurement of the elongation speed of RNA polymerase II showed in the genes containing these exons. The speed of transcription is in fact reduced when DBIRD is absent. "So the speed of RNA polymerase II directly influences the inclusion or exclusion of exons during the splicing process", continues Pierre Close.

Establishing links with pathology

To summarize: in the presence of DBIRD, the elongation speed of RNA polymerase II increases which causes the exclusion of exons rich in A/T of the final messenger RNA. On the other hand, in the absence of DBIRD,
as the RNA polymerase II is slower, it has the time to transcribe the exons rich in A/T and these are therefore included in the mature messenger RNA.

The discovery by the researchers of this new mechanism which regulates alternative splicing of mRNA by intermediary of transcription by RNA polymerase II was rewarded by being published in the journal *Nature* (1).

Another important aspect of this study is the presence of the DBC1 protein within the DBIRD complex. "This protein emanates from a gene which is in an impaired or absent locus in some breast cancers", explains the scientist. "*DBC1 plays a part in some other types of cancers such as cancer of the esophagus or prostate*", adds Pierre Close. "*In addition to the discovery of a mechanism which links splicing to transcription, our study brings forth new information concerning the function of the DBC1 protein as well as the description of the ZIRD protein, which was unknown until now*," he continues. The next step that Pierre Close and his colleagues at the Medical Chemistry Unit are going to take is to place these discoveries in a patho-physiological context. Establishing links between DBIRD, and therefore DBC1 and ZIRD, and possible pathologies, will make it possible to define whether this protein complex-or the proteins that it is composed of- could constitute an interesting target from a therapeutic point of view to fight these diseases.