Chapter Eight

Transcriptional slippage: general discussion
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Summary

Studies on the transcriptome have been highly facilitated by next generation sequence technologies. The potential to detect unknown and rare transcripts is one of the advantages of this new technology. In chapter 4 we presented a unique ultra-deep sequencing approach to study tissue-specific alternative splicing events of the SGCE gene. We detected several novel low frequency variants that we further characterised in chapter 7. Most of these variants can be attributed to conventional alternative splicing except for two. These were variations affecting multiple exons and intra-exonic deletions (Figure 1). Both type of variants lack canonical splice sites, but do show short identical sequences of 1 to 8 nucleotides at the junction. We identified these low frequency variants in different genes, tissues, and species. We excluded that they represent an artifact of PCR, reverse transcription, or sequencing. The expression profile of the two novel events showed tissue-specific trends: they were predominantly expressed in the brain and muscle tissue and to a lower extent or even absent for one gene in blood. Intramolecular transcriptional slippage is the most plausible mechanism that can explain these events. In this model, the pre-mRNA molecule dissociates from the DNA template, followed by reannealing, which can occur at the same (intramolecular slippage) or at a different template (intermolecular slippage). The position at which reannealing occurs is probably dependent on the presence of short identical sequences. Intramolecular slippage is a process similar to intermolecular slippage. The latter is thought to generate chimeric RNAs. This was the first study reporting intramolecular transcriptional slippage. Here we discuss and speculate about implications of transcriptional slippage.

Figure 1. Illustration of variations affecting multiple exons and intra-exonic deletions. Both variants did not exhibit canonical splice sites, but short identical sequences at the junctions. Variations affecting multiple exons covers two observations: (A) deletions of parts of adjacent exons, including skipping of 0 to 2 exons and (B) deletion of part of the 3’ end of an exon, followed by skipping of 1 to 3 exons and a 5’ extended exon. Intra-exonic deletions describe small deletions within one exon. The range of deletions was highly variable and skipping of 14 to 158 nucleotides was observed for variations affecting multiple exons and 6 to 100 nucleotides for intra-exonic deletions.
Discussion

Transcriptional slippage – is it functional or a side-effect of a highly dynamic transcriptional regulation?

Regulation of gene expression is controlled by several mechanisms such as chromatin condensation, DNA methylation, transcriptional initiation, mRNA stability, or post-translational modifications. All these mechanisms create a highly dynamic transcriptional regulation, which is essential to facilitate a response to environmental changes. As discussed in chapter 7, transcriptional slippage may be controlled by sequence variations, secondary structures, or stress situations, but it may also be a product of this dynamic transcriptional regulation. For instance, pausing of the RNA polymerase II complex can be a slippage-inducing factor. The property to pause and reactivate transcription has been implicated as a means to regulate transcription by providing the opportunity for coordination of co-transcriptional events and it is thought to facilitate transcription through nucleosomes. The regulated pausing and release of the RNA polymerase II complex was mainly observed at promoter-proximal regions in early elongation, but a recent study observed pausing throughout the body of all mRNAs. As a consequence of the promoter-associated pausing, upstream slippage of 2, 4, or 6 nucleotides was observed in a stretch of three CU-repeats followed by reannealing and elongation in vitro. Pausing is mainly induced by nucleosomes, suggesting that epigenetic mechanisms like histone modifications can induce transcript dissociation, for instance when a gene switches from an active to an inactive state. In this line, dissociation of the transcript can act as a mechanism to regulate gene expression by halting its transcription. On the other side, dissociation of the transcript can also be a side-effect of pausing of the RNA polymerase II complex similar to the mechanism observed at promoter-proximal regions. In both cases, deletions caused by reannealing is likely a side-effect.

Irrespective of the underlying mechanism, most dissociated transcripts do not form functional mRNA molecules and will be degraded. Our data suggest that some of these dissociated transcripts reanneal and are further elongated resulting in transcripts carrying deletions. Transcripts with frameshift deletions are likely to be targeted by nonsense-mediated decay. However, a considerable proportion of deletions did not alter the reading frame (SGCE: 24%; POLR2G: 36%; SLC25A3: 47%) and are therefore likely to be translated. Their impact or contribution to the proteome remains elusive.

Transcriptional slippage and disease

Strand slipping also occurs at the DNA level. It is a common cause of sequence expansions in disorders involving trinucleotide sequence repeats such as Huntington’s disease. During DNA replication the template strand can loop out resulting in deletions...
or the newly synthesised strand can dissociate and reanneal forming a loop resulting in insertions.

It is not known whether transcriptional slippage plays a role in disease. We observed low levels of transcriptional slippage in healthy controls. Factors leading to a less tight association of the RNA-DNA hybrid may result in enhanced slippage and accumulation of slippage products, which can lead to disruption of the cell homeostasis and cellular stress. Transcriptional slippage events resulting in proteins with toxic gain-of-functions may cause or predispose to diseases. Also, chimeric RNAs that do not underlie chromosomal aberrations such as intermolecular slippage products can play a causative role in cancer development.\textsuperscript{12,13}

Assuming a role for slippage in disease, the question of inheritability emerges. As slippage occurs at the transcriptional level, we only expect an effect on the next generation, when a genetic component that affects factors controlling slippage can be passed on to the next generation and not the altered mRNA molecules themselves. These factors include sequence variations or factors influencing either the local rate of transcription (transcriptional pausing) or affecting the strength of the RNA-DNA hybrid, implying that the affinity of transcript slippage can be inherited to certain extent.

**Transcriptional slippage - tissue-specific trends**

We investigated transcriptional slippage events in three tissues, brain, heart, and blood. Most slippage products were observed in brain and muscle tissue. In blood, they were present to a much lower extent and even absent in one of the genes tested.\textsuperscript{10} There are two possible explanations for our observations. The reduction or lack of slippage products in blood may be explained by the different life spans of different cell types. Leukocytes have a high turnover rate and are constantly renewed by the stem cell pool. Their life-span ranges from a few days to a few weeks, depending on the type of leukocyte.\textsuperscript{14} In contrast, cardiac muscle cells as well as neurons exist for a lifetime. It is generally believed that cardiomyocytes do not proliferate\textsuperscript{15} or only to a small extent.\textsuperscript{16} Also, there is little or no continuous production of neurons.\textsuperscript{17} These observations suggest that slippage occurs in mature or aging cells. If this hypothesis is true, one would expect enhanced slippage in elderly individuals, but we observed a similar rate in brain and muscle cells of a 7- compared to a 77 years old individual. These results suggest that transcriptional slippage occurs at low frequencies in all cell types, but more often in some tissues. In blood, slippage may be masked or reduced due to a constant renewal of cells and/or the presence of immature cells. Different tissues with different turnover rates, but also the same tissues of a fetus, or different developmental stages need to be studied in order to draw a conclusion.
Another possible explanation for tissue-specific differences is of more philosophical nature. Our observations raise the question, whether slippage is a product of a more “sloppy” and uncontrolled transcriptional regulation in brain and muscle versus blood or whether there is a less complex transcriptional regulation in blood compared to a more complex, dynamic, and plastic regulation in brain and muscle tissue. The brain is one of the tissues that developed most during evolution, which can account for both hypotheses. Neural plasticity facilitates remodelling and rewiring of the brain, explaining why the brain significantly differs among individuals. Also muscle is a plastic tissue, which is able to adapt to changes, however, to a lower extent. Muscular adaptations range from an increased muscle size to changes in contractile characteristics. One major difference to blood is probably the overall expression level of proteins; enhanced transcription may lead to enhanced slippage. Transcriptional differences are reflected in the high levels of alternative splicing observed in muscle and especially brain compared to blood. Also the number of genes expressed in blood is rather low.

We cannot conclude whether slippage is a product of a more controlled or less strict regulation or due to a higher rate of transcription in brain and muscle. Either way, our observations reflect tissue-specific differences in transcriptional regulation, which can be an unwanted by-product or a functional means of transcriptional regulation.

**Studying transcriptional slippage**

Studying transcriptional slippage is limited by the low frequency of slippage products. Whole transcriptome sequence approaches will not be sensitive enough to detect reported rare events. To our knowledge, our study is the first study performing ultra-deep amplicon sequencing of single genes with a depth of 10,000 sequence reads per amplicon, illustrating the resolution required to detect slippage products.

We did not detect slippage events involving large intronic regions. Slippage occurs during transcription on pre-mRNA level, thus still containing intronic sequences. Intramolecular slippage is also likely to occur within introns and between exons and introns. Slippage events within introns will not be detected since the transcript will still have intact splice sites and thus appear as wildtype transcripts after splicing of respective introns. Slippage products affecting exons and introns will only partly be detected (Figure 1B); they result in large amplicons that will not be amplified with PCR conditions used in our study (optimised for ~500-bp). Studying intermolecular slippage events such as chimeric RNAs remains a challenge; our sensitive PCR-based approach only allows detection of intramolecular transcriptional slippage events.
Conclusion and further perspectives

We identified intramolecular transcriptional slippage in healthy individuals at low frequencies. Little is known about transcriptional slippage; the proposed mechanisms are largely speculative. We hypothesise that slippage can be part of, or a by-product of a highly dynamic transcriptional regulation. Enhancement of transcriptional slippage as well as certain slippage products, such as chimeric RNAs or toxic gain-of-function products, can have a deleterious effect and thus play a role in disease. We observed more slippage products in brain and muscle compared to blood, which may be a result of different turnover rates of different cell types, of a more dynamic transcriptional regulation in brain and muscle, or of lower overall protein expression levels in blood. More genes and tissues or cell types at different developmental stages should be tested in order to draw conclusions.

We identified several recurrent slippage events in our study, which implicates that slippage does not occur at random and that certain factors induce or favour slippage, such as “common” sequences at the junction. Bioinformatic approaches can be applied to search for sequence variants or motifs involved in frequently observed events or recurrent sites of slippage. Another interesting field of research is whether more transcriptional slippage events are found in diseased tissues or cancer cells, as well as the question whether cellular stress induces slippage or whether enhanced slippage induces cellular stress.

References

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