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Minor variation in the 3' downstream region of *eGFP* reporter gene substantially increases level of its expression in mouse and human cells

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Motivation and Aim: The process of transcription termination appears to be complex and provides one of the levels of gene regulation [1]. We explored the influence of transcription termination defect on the level of *eGFP* reporter mRNA in transiently transfected mouse, human and *Drosophila* cell cultures.
Methods and Algorithms: We generated "wild type" and mutant double-reporter plasmids encoding mCherry (inner reference) and eGFP (reporter) fluorescent proteins. In the mutant constructs, a point deletion was introduced shortly (32 nt) downstream of the AAUAAA polyadenylation signal (PAS) of the *eGFP* reporter. Using RT-qPCR and FACS analyses, we determined the reporter mRNA and protein levels in transiently transfected human HEK293T, mouse C57BL/3T3-LCD, and *Drosophila* Kc167 cultured cells. We obtained the C57BL/3T3-LCD cell line by immortalization of primary mouse embryonic fibroblasts isolated from C57BL/6J embryos according to [2]. 3′-RACE method [3] was used to identify the 3′ end(s) of the mature *eGFP* mRNA molecules in HEK293T cells.
Results: We found that the one-nucleotide deletion introduced shortly downstream of the PAS causes up to 4-fold increase in both mRNA and protein production in transiently transfected human and mouse, but not in *Drosophila* cells. This deletion of a single C 32 nt downstream of the PAS leads to twice more frequent cleavage of pre-mRNA molecules within 14 nt downstream of the PAS (85 % of cases vs 43 % in the control), whereas the wild-type pre-mRNA molecules are typically cleaved more distally: at 31 nt downstream of the PAS and a number of other sites.
Conclusion: Even a small change in the region immediately downstream of 3′ UTR, which is not present in the mature mRNA, can substantially affect the expression level of the upstream gene. The results indicate the great regulatory potential of the 3′ downstream region, which also may be applied in biotechnology.
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