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Activating and Imaging Gene Expression in Single Cells Using Light

In our previous work, we devised a single-cell approach where transcription could be observed in living cells in real time by using a direct fluorescent read-out of a reporter RNA. A doxycycline inducible gene could be induced to synthesize a transcript containing stem-loops derived from the RNA phage MS2. Transfection of this cell line with a fluorescent fusion protein of MS2, a high-affinity RNA binding protein specific for the stem-loops provided real-time monitoring of transcription. Using this system combined with high speed imaging has revealed the time course of transcription of nascent mRNA molecules containing this stem-loop, its release into the nucleoplasm and its diffusion into the cytoplasm¹. However, the induction of this gene occurs in all cells exposed to doxycycline. For the purpose of investigating and precisely controlling gene expression in individual cells, particularly within tissues, it would be desirable to activate a reporter gene using only photons, thereby spatially confining gene expression to a single cell². This has been achieved by preparing a chemically altered transcription factor that is unable to activate a reporter gene until it is exposed to photon excitation³. We developed a caged variant of the steroid transactivator, PON A that is unable to bind the ecdysone receptor and hence the response element unless the blocking group is photolysed by UV light. In cell cultures containing the reporter gene, we were able to activate transcription using short laser pulses. When the promoter was multimerized to consist of 85 repeats of the response element, the transcription of RNA can be initiated rapidly, within minutes. Synthesis of specific fluorescent proteins from the reporter RNA validated gene expression. We have now synthesized a caged PON A that has a two-photon cross section. Currently we are inserting this gene into a lentivirus vector so that it can be introduced into tissues for intravital imaging of gene expression.

Publications

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