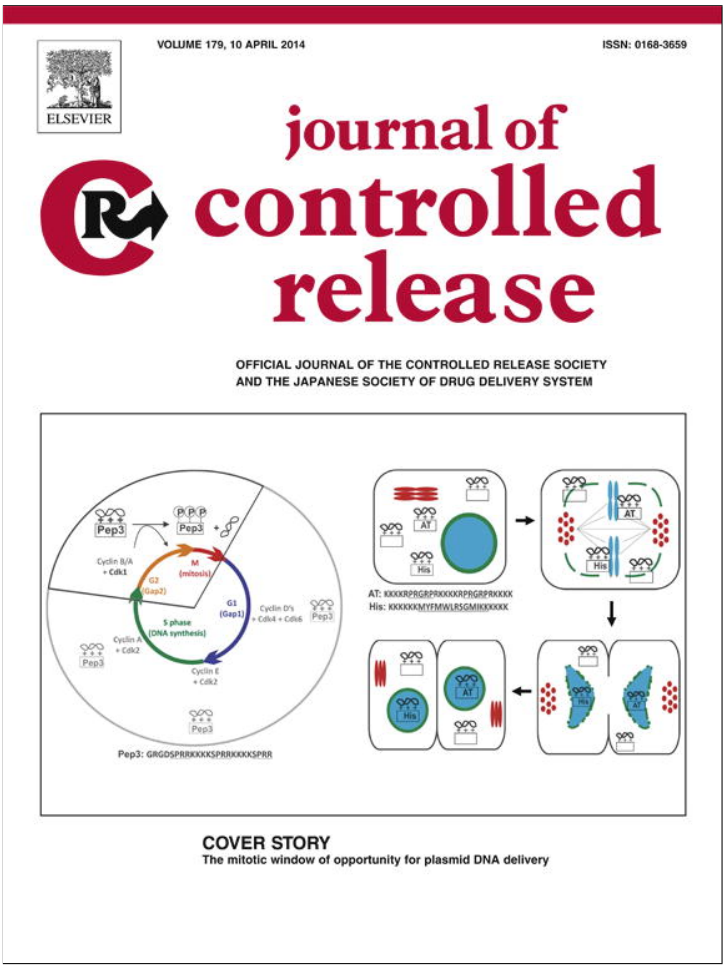

Journal of Controlled Release 179 (2014) 76

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Cover story

The mitotic window of opportunity for plasmid DNA delivery

The nucleus of eukaryotic cells contains the entire human genome, protected by a double-membrane organized around the chromosomes. The main function of the nucleus is to protect the genetic material and to control cellular activities by regulating gene expression. There is a tremendous interest in using plasmid DNA to induce the expression of therapeutic genes into patient's cells. Although the concept of bringing engineered plasmids into the nucleus is straightforward, the delivery of plasmid DNA to this highly protective environment has been puzzling scientists for decades. Initial attempts to bring plasmid DNA into the cell's nucleus tried to overcome the nuclear barrier by traversing the nuclear pore complexes (NPCs). NPCs have a central channel with a diameter of about 9 nm, which can be enlarged to about 30 nm upon active transport. Naked DNA is believed to cross these nuclear entry ports by snake-like movements, although the efficiency of crossing the NPCs remains rather low. As a result, non-dividing cells are still difficult to transfect [1].

Another option for plasmid DNA to gain access to the nuclear interior is during the so-called mitotic window of opportunity [2]. A window of opportunity is a short time period during which an otherwise unattainable opportunity exists. In this case, the short time frame consists of the time needed for the cell to go through mitosis. During cell division, the nuclear membrane is temporarily disassembled and nucleoplasmic and cytoplasmic components can mix with each other. In this way, a fraction of the cytoplasmic plasmid DNA can end up in the newly formed daughter nuclei when cell division is completed. After that time, the only access to the nuclear interior is again through the NPCs.

In this issue, the article by Remaut et al. [3] evaluated two new strategies to maximize the delivery of plasmid DNA during this mitotic window of opportunity. To do so, they explored viral ways of hitchhiking the cellular machinery. A first important consideration is that plasmid DNA present in the cytoplasm will eventually degrade into inactive smaller DNA pieces. Thus, exposing the plasmid DNA in the cytoplasmic environment only during cell division would be beneficial to achieve a high concentration of active plasmid DNA at the start of mitosis. By incorporating the recognition sequence of cyclin-dependent kinase-1 (CDK1) (S/TPXK/R) in plasmid DNA containing nanoparticles, they obtained DNA nanoparticles which are specifically phosphorylated at the onset of cell division. This phosphorylation introduces negative charges in the peptide backbone which results in the specific release of plasmid DNA in dividing cells. When compared to peptides lacking the phosphorylation sequence, a small, but statistically significant better transfection was observed in dividing cells.

In the second strategy, the authors explored ways of anchoring plasmid DNA nanoparticles to the chromatin which is exposed during cell division. In this way, retention of plasmid DNA in the newly formed nuclei can be assured, as the newly formed nuclear envelopes will always enclose the chromatin material and hence the plasmid DNA or nanoparticles attached to it. As chromosome binding factors, AT-hooks with consensus motif PRGRP were chosen, as well as the consensus sequence from the latent DNA herpes virus LANA (latency-associated nuclear antigen). The *in vitro Xenopus* nuclear assembly reaction clearly demonstrated better chromatin targeting using these sequences, but the chromatin targeting proved more difficult to achieve in living cells.

The difficulties described in the study by Remaut et al. provide valuable lessons. Although the approach is sound, the results do not match the expectations. This means that there are many other factors that the authors did not consider, or the assumptions taken may not be the right ones. While the experiments need to be done by simplifying complex problems into an experimentally manageable one, such a process may unintentionally delete yet-unknown critical factors. Advancing science takes time, and requires unraveling the complexity of the intracellular environment step by step. The result of the study by Remaut et al. made one small step forward in knowledge, but the authors' attempt to describe the difficulties and problems made one large leap forward. It is time for many of us to stop highlighting only a marginal improvement, and discussing the real challenges to define the problems more clearly so that they can be solved.

References

[1] M.A.E.M. van der Aa, E. Mastrobattista, R.S. Oosting, W.E. Hennink, G.A. Koning, D.J.A. Crommelin, The nuclear pore complex: the gateway to successful nonviral gene delivery, Pharm. Res. 23 (2006) 447–459.
[2] N. Symens, S.J. Soenen, J. Rejman, K. Braeckmans, S.C. De Smedt, K. Remaut, Intracellular partitioning of cell organelles and extraneous nanoparticles during mitosis, Adv. Drug Deliv. Rev. 64 (2012) 78–94.
[3] K. Remaut, N. Symens, B. Lucas, J. Demeester, S.C. De Smedt, Cell division responsive peptides for optimized plasmid DNA delivery: the mitotic window of opportunity? J. Control. Release 179 (2014) 1–9.

Kinam Park
Purdue University
Departments of Biomedical Engineering and Pharmaceutics
West Lafayette, IN, USA
E-mail address: kpark@purdue.edu

0168-3659/$ – see front matter
http://dx.doi.org/10.1016/j.jconrel.2014.03.004