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NEWS AND COMMENTARY

Communicating RNA

Commenting on communicator RNA

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Harking back to the 'RNA world' that is considered to be the beginning of life about 4.2 billion years ago,[1,2] RNA (ribonucleic acid) molecules recapitulate all biological activities necessary for life: containment of genetic information (for example, messenger RNA (mRNA)), regulation of gene expression (small-interfering RNA and micro RNA), scaffolding of tri-dimensional structures (for example, transfer RNA), enzymatic activities (for example, ribosomic RNA), storage of energy (for example, adenine and guanine in their triphosphate form) and protection of an organism's integrity by stimulating host defence mechanisms (immunostimulating RNA).

Another fundamental feature of pluricellular living is communication between cells. The presence of abundant and highly efficient RNases in the intercellular space and body fluids has led scientists to consider improbable the existence of functional extracellular naked RNA in organisms. This notion should, however, be challenged in the light of several pieces of experimental data, including those presented by Diken *et al.*[3] in the July 2011 issue of *Gene Therapy*. The authors document that naked mRNA injected into the lymph nodes of mice is taken up by phagocytic cells through macropinocytosis. This can be recapitulated *in vitro* using human and mice phagocytes, such as dendritic cells or macrophages. Similar phenomena were documented more than 20 years ago when Wolff *et al.*[4] reported that intradermal injection of naked mRNA in mice resulted in local protein expression and when Gilboa and colleagues[5] reported that co-incubation (so called 'passive pulsing') of mRNA with human dendritic cells resulted in the presentation of major histocompatibility complex (MHC)-associated peptides derived from the antigen encoded by the mRNA. We further documented that resident cells in mouse and human dermis take up locally injected naked mRNA in a saturable and calcium-dependant way.[6] In these previous works, it could be shown that the uptake process is active: RNA molecules do not simply diffuse through membranes but are phagocytosed and transported to the cytosol in an (as yet) unknown way. Thus, mRNA molecules prejudiced as very labile in the RNase-contaminated extracellular milieu are surprisingly functional after penetrating local cells adjacent to the site of their delivery.

To date, no experimental data are available to explain the capacity of exogenous RNA to survive and then penetrate cells before being degraded by RNases. Underlying this unexpected observation could be the pyrimidine-specificity of extracellular RNases,[7] which may lead to relative stability of purine-rich RNA and of RNA molecules with pyrimidine bases protected within three-dimensional structures. Alternatively, cationic proteins such as anti-viral peptides (for example, LL37) eventually present in the intercellular milieu could complex and stabilise the injected mRNA before it is degraded by RNases.[8]

Up to now, cross presentation has been thought to rely on the uptake of exogenous antigens in the form of protein. Although this format may be appropriate for MHC class II antigen presentation, it is not optimal for the presentation of therapeutically relevant endogenous MHC class I-associated peptides.[9] Indeed, the set of MHC class I-associated peptides made from endogenous, that is intracellularly translated proteins can be distinct (though overlapping) from the set made from exogenous proteins.[10] As shown in the study by Diken *et al.*,[3] phagocytes such as antigen-presenting cells (APCs) are particularly efficacious in taking up RNA from the extracellular space. This would allow APCs to cross present through the MHC class I pathway exogenous antigens expressed after uptake of extracellular mRNA, either injected or released from dying cells. Phagocytosis by APCs of mRNA released by neighbouring cells that die because of infection or endogenous dysfunction (chromosomal damage for example), may be of importance for the MHC class I presentation of antigens derived from viruses that cannot infect APCs[11] and tumor-specific antigens, respectively. No data are yet available to quantify the relative importance of protein uptake versus mRNA uptake for immune (cross-) presentation.

Furthermore, it can be envisaged that the spontaneous uptake of recombinant naked mRNA by phagocytes *in vitro* as well as by phagocytes (for example, lymph node-resident dendritic cells[3]) and other cells (for example, skin fibroblasts[6]) *in vivo* at a site of injection, reflects an ancestral biological mechanism that uses naked RNA as a communicator between cells. As RNA is chemically very stable compared with double-stranded DNA or proteins at acidic pH for example, it could have been the ideal communicator between neighbouring or even distant cells at early times of evolution. Beyond immune cross-presentation as mentioned above, it can be speculated that specific (selective or induced) or non-specific (cell death) release of naked RNA (micro RNA or mRNA for example) by cells and uptake by neighbouring cells could be a very controlled and precisely coded pathway for intercellular communication. Although the capacity of sorted RNA contained in exosomes to serve as a communicator between neighbouring as well as distant cells and tissues is presently attracting much attention,[12,13] our knowledge about the existence and physiological relevance of naked RNA as a local communicator RNA is in its infancy.

After uptake by macropinocytosis as shown by Diken *et al.*,[3] translocation of

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large RNA molecules from endosomes to the cytosol has to be achieved. Disruption of the endosome's membranes, encapsulation into exosome structures followed by release and re-uptake or selective export of RNA across the endosomal membrane could be proposed as mechanisms. Further work is required to determine the mechanisms actually involved.

The unexpected capacity of injected naked mRNA to be internalised and efficiently translated by local cells including APCs and then to prime specific immune responses, has started the hunt for what may well turn out to be to a wealth of biologically important roles for naked communicator RNA (CoRNA).

CONFLICT OF INTEREST

The author declares no conflict of interest.

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