A glass bead game

Thomas M. Bayerl

Microscopic glass beads coated with lipid membranes provide a sensitive detector of interactions between proteins and ligands. The changing spatial order of the array of beads in solution is the key.

At the start of a game of snooker, the balls are arranged in an ordered shape, each touching its neighbours, in a triangular rack. As the game begins, a player aims the cue ball at the apex of the triangle and disperses the object balls over the table. The sudden change in order, triggered by the transfer of momentum from the cue ball, is a kind of (irreversible) 'phase transition', from a condensed, ordered state to a dispersed, disordered one. No one would imagine that the object balls could take on their initial triangular arrangement spontaneously, just by being dropped all at once onto the billiard cloth. Nor would they think that the phase transition could be achieved without transferring momentum from the cue ball. But, in their paper on page 139 of this issue, Baksh *et al.*[1] suggest that, with billiard balls shrunk to the size of microscopic beads and the billiard cloth replaced by a smooth glass or plastic surface, this is exactly what can happen.

The spontaneous self-assembly of a disordered bunch of microscopic glass (silica) beads dispersed in water into highly ordered, two-dimensional colloidal crystals — akin to preparing a snooker game without needing the triangular rack — is already the subject of intense research. Initiating a phase transition in the colloid without adding external momentum (the cue ball) or thermal energy is, however, a new achievement. Yet the only additional ingredients are simple: the surfaces of the beads are coated with an ultrathin organic film, and molecules designed to interact with this film are dissolved in the surrounding aqueous solution.

Through a light microscope, Baksh *et al.*[1] saw that silica beads, 5 μm in diameter, each encased in a shell of 5-nm-thick phospholipid bilayer, form two-dimensional colloidal crystals spontaneously. The degree of order in the colloidal crystals depended on whether or not ligands in the bilayer could bind to proteins (receptors) dispersed in the surrounding solution. The choice of a phospholipid bilayer as the bead coating is not accidental. Lipid bilayers are the basis of all biological membranes. When applied as a coating to glass beads, these so-called supported bilayers have a distinct advantage, compared with covalently bead-bound molecules or self-assembled monolayers — they retain the fluidity and functionality of a natural membrane and can accommodate ligands or receptors without loss of their functional activity. Since the first supported bilayer was fabricated[2] in 1985, they have been studied extensively, and have been applied in biosensors[3], in advanced biosepa-ration techniques[4] and in high-throughput molecular screening[5].

A series of time-sequence images taken by Baksh *et al.*[1] (Fig. 3a on page 140) reveals that the colloidal crystals can be disrupted, just like billiard balls: there is a phase transition from a condensed to a dispersed state when receptor protein is added (Fig. 1). The forces involved are essentially van der Waals attractions between the beads, and the attractive and repulsive electrostatic forces that arise from both the bead surface and the bilayer. The binding of the receptor to the ligand at the bilayer surface modulates the 'pair interaction potential' between adjacent beads, thereby disrupting the condensed phase in a sudden transition. As a result, binding of the receptor can be quantified in a straightforward way, by calculating a 'pair distribution function', which encapsulates how close and ordered the beads are in each state. By adding different proteins, and even complex protein mixtures, to the solution, Baksh *et al.* proved that the transition from ordered to dispersed state is triggered only if specific binding between the bilayer-attached ligand and the dissolved receptor occurs. In contrast, the presence of non-specific, or even competitive, binders did not result in a phase transition — but neither did it diminish the response of the system to the specific receptor.

The science of this technique is intriguing; using it to detect specific protein-binding events, without the need to use labels such as fluorescent proteins, is an interesting prospect. The method works at very low concentrations (in the pico- to nanomolar range), and could even be automated for screening purposes. The collective nature of the phase transition makes it easy to watch through a microscope, but direct observation is not necessary. Instead, the transition can be detected by analysis of the dispersed phase, by calculating its pair distribution function. Automated screening could be carried out using microwell plates with transparent bottoms, into which ligand-coated beads could be pipetted in the presence of potential receptor proteins. After allowing the beads to settle, the pair distribution function could be read out from an automated imaging system.

In his novel *The Glass Bead Game*, Hermann Hesse never described explicitly how the game worked. Similarly, Baksh *et al.*[1] do not offer a comprehensive description of the molecular mechanisms underlying the phase transition in their own glass bead game. Neither do they elaborate on how different receptors might manifest themselves through distinguishable pair distribution functions. But, as the scholars in the novel devoted their lives to the endless ramifications of the glass bead game, this work by

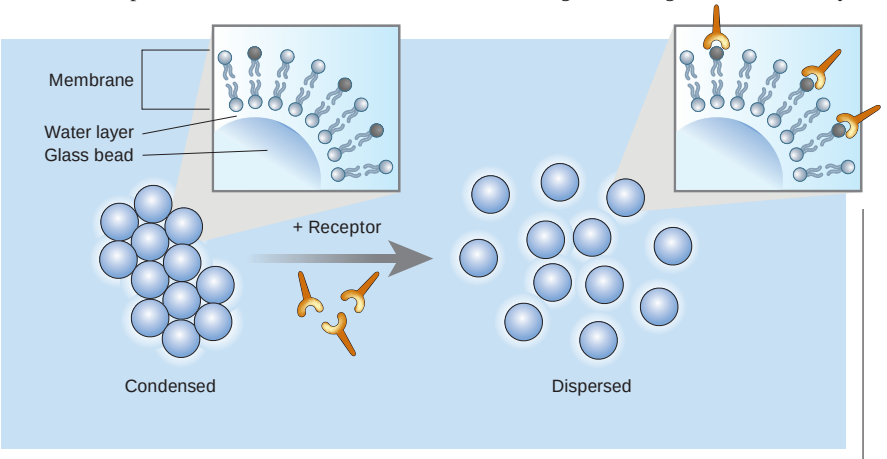

Figure 1 **In an aqueous solution, microscopic glass beads, each coated with a lipid membrane, assemble into an ordered two-dimensional crystal. Baksh *et al.*[1] show how that order can be disrupted by adding to the solution a receptor protein that binds to a ligand at the membrane surface. This dramatic transition from an ordered to a dispersed state could be built into an automated scheme of detection for protein–ligand interactions.**

Baksh *et al*. may open the door to the automated characterization of a wide range of complex molecular interactions that are at present poorly understood. ■

Thomas M. Bayerl is at the Physical Institute, University of Würzburg, Am Hubland, 97047 Würzburg, Germany.
e-mail: bayerl@physik.uni-wuerzburg.de

1. Baksh, M. M., Jaros, M. & Groves, J. T. *Nature* **427,** 139–141 (2004).
2. Tamm, L. & McConnell, H. M. *Biophys. J.* **47,** 105–113 (1985).
3. Sackmann, E. *Science* **271,** 43–48 (1996).
4. Loidl-Stahlhofen, A., Kaufmann, S., Braunschweig, T. & Bayerl, T. M. *Nature Biotechnol.* **14,** 999–1002 (1996).
5. Loidl-Stahlhofen, A. *et al. Adv. Mater.* **13,** 1–6 (2001).

Stem cells

How to make eggs and sperm

M. Azim Surani

Embryonic stem cells can develop into many specialized cell types in culture dishes. It now seems that they can also generate primordial germ cells, which then go on to form sperm and eggs.

A fertilized egg is potentially immortal: this fusion of egg and sperm gives rise not only to a new individual, but also (theoretically at least) to an endless series of generations. Three groups now suggest that it is possible to generate both of these remarkable cells — known collectively as germ cells — in a culture dish. Geijsen and colleagues[1], writing on page 148 of this issue, and Toyooka *et al.*[2], writing in *Proceedings of the National Academy of Sciences*, describe how they obtained sperm-like cells from mouse embryonic stem cells (ES cells) *in vitro*. Geijsen *et al.* even discovered that injecting their sperm-like cells into natural mouse eggs resulted in early embryonic development. Meanwhile, Hübner *et al.*[3] reported earlier this year in *Science* that they have succeeded in obtaining egg-like cells from mouse ES cells. So it is possible to produce germ cells with at least some attributes of sperm and eggs *in vitro*. These findings raise the possibility of deriving similar germ cells from human ES cells in culture — an idea that raises ethical issues as well as the prospect of unprecedented medical advances.

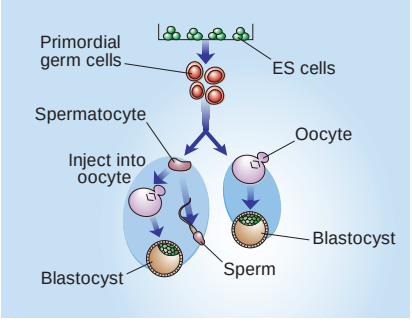

Figure 1 **Germ cells from stem cells. In two of the new papers[1,2], embryonic stem cells (ES cells), cultured as aggregates, formed structures resembling early embryos ('embryoid bodies'); in a third paper[3], the aggregates were permitted to undergo spontaneous differentiation. In all cases, a proportion of the ES cells produced primordial germ cells. Left, Geijsen *et al.*[1] found that some of these cells differentiated into spermatocytes (precursors of sperm) in the embryoid bodies. Moreover, injecting the spermatocytes into unfertilized eggs led to development to the early, blastocyst stage. Toyooka *et al.*[2] found that culturing primordial germ cells with cells from fetal testis also produced sperm. Right, Hübner *et al.*[3] isolated, re-aggregated and cultured primordial germ cells, which formed complex structures within which were found developing eggs. Release of these oocytes from the surrounding cells led to spontaneous activation and development to the blastocyst stage.**

ES cells derived from five-day-old mouse or human embryos ('blastocysts') have the exceptional potential — depending on the culture conditions — to either multiply indefinitely or develop into an array of specialized cells[4]. To try to persuade mouse ES cells to generate eggs and sperm (Fig. 1), Geijsen *et al.*[1] and Toyooka *et al.*[2] allowed aggregates of the cells to differentiate into structures that somewhat resemble early embryos; Hübner *et al.*[3] allowed ES-cell aggregates to undergo random differentiation spontaneously. The result was that, in the embryo-like structures and among the randomly differentiated cells, there were cells resembling primordial germ cells, which the authors detected by the expression of certain marker genes. The authors then isolated some of these primordial germ cells — the precursors of sperm and eggs — and allowed them to proliferate in culture. Curiously, all three groups noticed that the gene-expression pattern of the primordial germ cells showed highly accelerated development. Developmental timers are normally exquisitely regulated, so it will be important to know why the timing went awry in these cases.

The next stage involved transforming the primordial germ cells into sperm or eggs, which is a complex process that, *in vivo*, occurs within specific microenvironments[5,6]. To achieve this *in vitro*, the groups adopted different approaches. Geijsen *et al.* allowed the process to occur spontaneously in the embryo-like structures; Toyooka *et al.* cultured the primordial germ cells with normal fetal gonadal cells. In the work by Hübner *et al.* the primordial germ cells were allowed to form aggregates again. Either sperm-like[1,2] or egg-like[3] cells were produced (possibly depending on the procedure used). At this time, the number of chromosomes must be halved to allow male and female germ cells to make equal genetic contributions at fertilization[7]. This apparently occurred, although additional confirmation would be desirable.

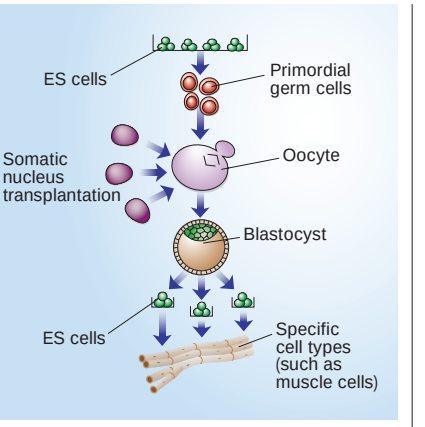

Figure 2 **A possible use for eggs derived from ES cells — nuclear transplantation. As shown in Fig. 1, oocyte-like cells could be made in culture from ES cells, by way of primordial germ cells[3]. After stripping the oocytes of their own genetic material, they could be used as recipients for nuclei from adult (somatic) cells such as skin cells. The somatic nucleus could then be 'reprogrammed' by factors present in the oocyte, which is then allowed to develop to the blastocyst stage. Blastocysts contain epiblast cells, from which new ES cells can be derived. Each type of ES cell will inherit some properties of the adult donating the somatic nucleus, such as a propensity for certain complex diseases. The ES cells could then be used to derive specific cells with which to study the progression of those diseases, and perhaps to generate treatments[10].**

Developing sperm and eggs must also acquire their characteristic identity tags, or 'imprints'[8], which regulate their complementary functions when embryonic development begins after fertilization. However, it is not yet known whether the eggs and sperm now generated[1–3] have the appropriate imprints. This information will be crucial, because sperm and eggs can seem normal even without the appropriate marks — but their functions will be affected after fertilization when development starts[8]. So, although Geijsen and colleagues did obtain blastocysts when they injected their sperm-like cells into unfertilized eggs, we cannot make any predictions about the long-term development of these early embryos. It is also interesting that the eggs generated by Hübner *et al.* developed spontaneously to the blastocyst stage once released from the surrounding cells — even though mature eggs should remain 'arrested' until they are fertilized or