Fertility after intact ovary transplantation

Frozen banking of whole organs for transplantation is starting to look feasible.

Transplantation surgery, which is limited by the supply and short-term viability of fresh donor organs, would be revolutionized if these could survive freezing, but early claims of cryopreservation[1] were never realized. Here we describe the successful transplantation in rats of ovaries, fallopian tubes and the upper segment of the uterus *en bloc* after storage in liquid nitrogen.

We anaesthetized adult female Lewis rats using sodium pentobarbital and removed the right ovary and the reproductive tract, with the ovarian vessels dissected to create short cuffs of aorta and vena cava[2,3]; these preparations were held in solution for less than an hour before transplantation. Syngeneic recipients were anaesthetized, heparinized and oophorectomized. The aorta and vena cava below the left renal artery were dissected for end-to-side aortic–aorta and veno–venous anastomoses using continuous suturing; the uterus was joined to the corresponding host segment. After paired operations lasting for 2 h, circulation was restored to donor organs (for further details, see supplementary information). All surviving recipients stayed healthy.

We used eight fresh organs for transplantation and also seven treated ones that we had perfused for 30 min at 0.35 ml min^{-1} with M2 medium containing 0.1 M fructose and increasing concentrations of dimethylsulphoxide (0–1.5 M). Following a protocol previously used for sliced ovarian tissue[4], we cooled the treated organs slowly in 5-ml cryovials (Nalgene), inducing ice nucleation at −7 °C in the modified chamber of an automated freezer (CryoLogic). After overnight storage in liquid nitrogen, we rapidly thawed the vials and removed the cryoprotectant by perfusion with a reversed concentration gradient (for further details, see supplementary information).

We monitored ovarian function by taking vaginal smears (see supplementary information) and by pairing some animals with stud males four weeks later. Ten weeks after surgery, we collected blood immediately after euthanasia to measure serum concentrations of follicle-stimulating hormone (FSH) and oestradiol-17β by radioimmunoassay and chemiluminescence, respectively. We prepared organs for histology and to assess ovarian follicle reserves.

All animals with fresh transplants reinitiated oestrus. They had normal numbers of ovarian follicles, including graafian stages (Fig. 1a), and uterine weights and FSH levels were similar to those of controls (9.7 ± 0.5 and 9.1 ± 0.4 ng ml^{-1}, respectively). Four animals with cryopreserved grafts (57%) had

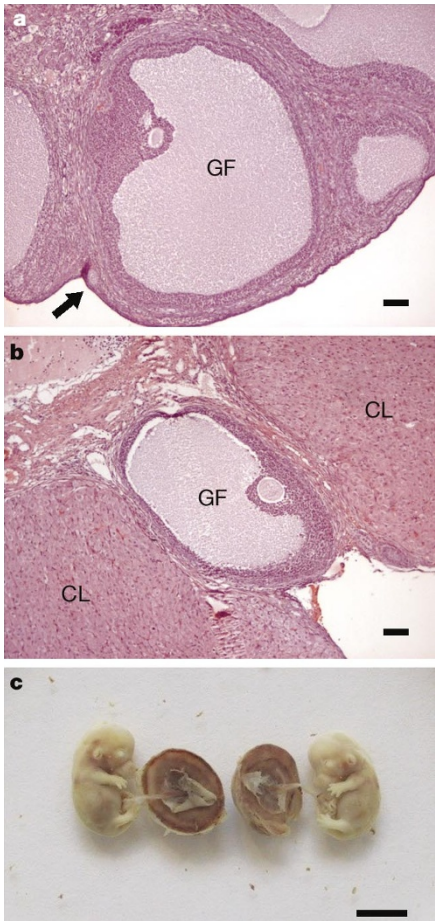

Figure 1 Transplantation success of ovaries and reproductive tract in rats. **a,** Fresh-ovary transplants had growing follicles (arrow) and mature graafian follicles (GF). **b,** Cryopreserved-ovary transplants were also follicular and contained corpora lutea (CL). **c,** Two fetuses conceived by one animal that had received a frozen–thawed transplant. Scale bars: 0.05 mm (**a**, **b**) and 5 mm (**c**).

follicular ovaries, and their corpora lutea indicated recent ovulation (Fig. 1b). One animal was pregnant, with two healthy fetuses implanted on either side of the uterine anastomosis (Fig. 1c). This group had higher serum FSH levels (21.3 ± 6.2 ng ml^{-1}), fewer follicles and lower oestradiol levels and uterine weights, but none was in the castrate range. Tubal and uterine morphology were indistinguishable from unoperated controls.

These results are encouraging, but indicate that ovarian function is compromised to some extent by freezing, perhaps because of intravascular ice formation — a problem that has previously frustrated attempts to cryopreserve kidneys[5]. Advances in vitrification may overcome this problem if the chemical toxicity of additives can be minimized[6]. Frozen banking of reproductive organs could eventually be useful in breeding from endangered species and as a fertility option for women and children who have undergone sterilizing chemotherapy[7,8]. Our success with ovarian transplants should stimulate investigation into the improvement of cryopreservation techniques for other organs.

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Chemistry

Amplification by compartmentalization

Autocatalysis and chemical amplification are properties of living systems that can lead to increased responsiveness and to self-replication. Here we describe a synthetic system in which a unique form of reagent compartmentalization gives rise to nonlinear kinetics that are subject to the precise size- and shape-selectivity of the host. The reactivity is reminiscent of autocatalytic behaviour[1], in which there is no direct contact between reagents and products, and our approach offers a general way to impose complex chemical behaviour onto synthetic systems.

Reversibly formed hosts, such as capsule **1** in Fig. 1 (ref. 2), temporarily surround smaller guest species and effectively divide a reaction milieu into two domains: reagents are either free in solution ('on'), where they react normally, or are encapsulated ('off'), where they are unreactive. The exchange of

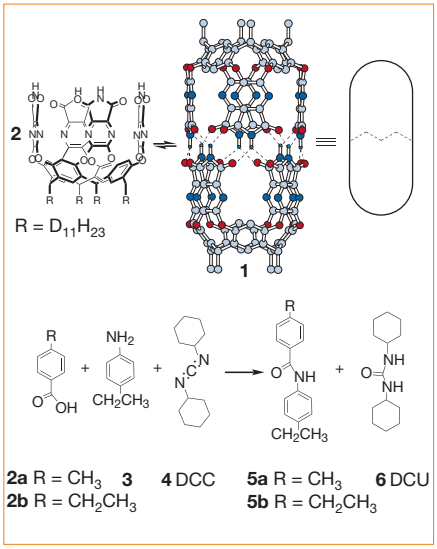

Figure 1 The dimeric capsule **1** encapsulates molecules of the proper size and shape and inhibits their reactivity. Reagent **4** and products **5a** and **6** are good guests, but the slightly larger product anilide **5b** is not. DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea.

guest species between these environments is thus a means of controlling reactivity.

Consider the reaction of *p*-toluic acid (molecule **2a** in Fig. 1) or *p*-ethylbenzoic acid, (**2b**) with *p*-ethylaniline (**3**) and dicyclohexylcarbodiimide (DCC; **4**). When 5 mmol each of **2a** or **2b,** 10 mmol of **3** and 1 mmol of **4** are mixed at 295 K in mesitylene, the almost identical anilides **5a** and **5b** are formed at almost identical rates, together with dicyclohexylurea (**6**) and *N*-acylurea byproducts.

These initial rates are much lower in the presence of stoichiometric amounts of **1**, which encapsulates **4** and ablates its reactivity. The initial, equilibrium concentration of free **4** is too low to be seen by nuclear magnetic resonance spectroscopy, and the reaction of **2a** or **2b** with the aniline proceeds only through these trace amounts of **4** in solution. In contrast to the kinetics in the absence of **1**, the reaction of the shorter acid **2a** is now much faster than that of the longer **2b** (Fig. 2). Moreover, addition of the shorter product **5a** accelerates the initial rate of the reaction involving **2a**, but addition of the only slightly longer product anilide **5b** has no effect.

This behaviour results from feedback loops in a self-regulating reaction cycle (Fig. 2a). The urea molecule **6** and toluic anilide (**5a**) — the products of the reaction — are both good guests for the host **1** (relative binding affinities: **5a**~**6** > **4**). Once formed, the products displace DCC from the capsule into the bulk solvent, where it can react with the acid. A single molecule of DCC reacts to yield one molecule each of urea and anilide, which each displace further DCC molecules, leading to the production of more urea and anilide in a chain

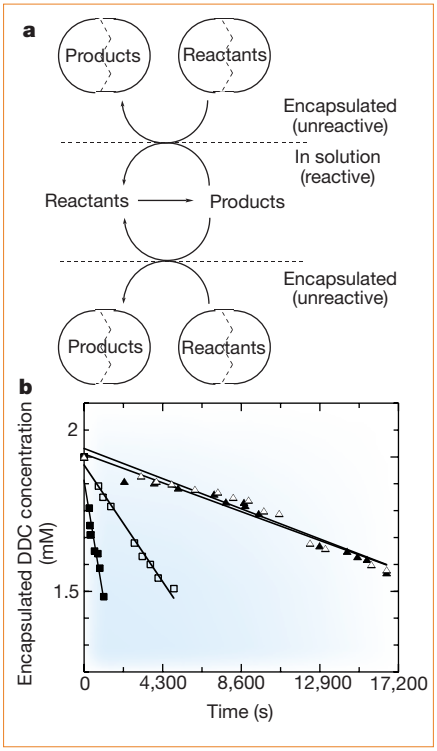

Figure 2 Reaction of compartmentalized reagents. **a,** For the reaction shown in Fig. 1, products **5a** and **6** displace reagent **4** from the capsule interior, creating a feed-forward reaction cycle. When acid **2b** is used, the slightly larger product anilide **5b** is a poor guest, and half of the reaction cycle is not operative. **b,** Dicyclohexylcarbodiimide (DCC) released from the capsule interior as a function of time for the reactions shown in Fig. 1. Initial concentrations: **4**, 2 mM; **1**, 2 mM; **3**, 10mM; **2a**, 2 mM (open squares) or 2 mM with 0.25 mM equivalents of amide **5a** (filled squares); **2b**, 2 mM (open triangles) or 2 mM with 0.25 mM equivalents of amide **5b** (filled triangles). Errors in integrations and concentrations are within 5%; straight lines are shown for guidance.

reaction. The capsule does not influence the reaction between acid and DCC — it merely limits the rate at which reagents encounter each other, giving rise to kinetics that accelerate with the increased concentration of free 'reactive' DCC.

The reaction of **4** with the very similar **2b** occurs with different kinetics because the product molecule *p,p′*-diethylbenzanilide (**5b**) cannot fit into the capsule[3]. Only one good guest — the urea molecule **6** — is generated in this instance, and no further reagents are released; the kinetics are not accelerated.

This behaviour, although easily understood, is difficult to classify. The kinetics have a sigmoidal character that depends on product formation, but the reaction is not autocatalytic as it has no true catalyst. Moreover, unlike templated autocatalysis, no direct contact is required between reagents and products — an advantage in that catalyst inhibition due to self-complementary products is not an obstacle. The nonlinear kinetics are viewed more correctly as an emergent property of the system as a whole, rather than of specific molecules within the system.

The combination of compartmentalization and molecular recognition does, however, result in chemical amplification, which is useful for increased sensitivity and self-replication. Other compartmentalized chemistry has similar potential, and product formation influences subsequent reactivity in zeolites[4]. The inherent benefits of compartmentalization are thought to be an important, if not essential, characteristic of living systems (for example, see ref. 5). As long as the relative timescales of guest exchange and reaction are appropriate, our approach can be generalized to a range of reactions and even to complex but well-defined chemical systems.

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COMMUNICATIONS ARISING

Palaeoclimatology

CO_2 and the end-Triassic mass extinction

The end of the Triassic period was marked by one of the largest and most enigmatic mass-extinction events in Earth's history[1] and, with few reliable marine geochemical records, terrestrial sediments offer an important means of deciphering environmental changes at this time. Tanner *et al.*[2] describe an isotopic study of Mesozoic fossil soils which suggests that the atmospheric concentration of carbon dioxide (pCO_2) across the Triassic–Jurassic boundary was relatively constant (within 250 p.p.m.v.), but this is inconsistent with high-resolution evidence from the stomatal characters of fossil leaves[3]. Here I show that the temporal resolution of the fossil-soil samples may have been inadequate for detecting a transient rise in pCO_2. I also show that the fossil-soil data are consistent with a large increase in pCO_2 across the Triassic–Jurassic boundary