Exponential DNA Replication by Laminar Convection

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It is shown that laminar thermal convection can drive a chain reaction of DNA replication. The convection is triggered by a constant horizontal temperature gradient, moving molecules along stationary paths between hot and cold regions. This implements the temperature cycling for the classical polymerase chain reaction (PCR). The amplification is shown to be exponential and reaches 100 000-fold gains within 25 min. Besides direct applications, the mechanism might have implications for the molecular evolution of life.

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Here we show that a temperature sensitive chain reaction can be driven by the temperature oscillation of a laminar convection cell. The molecules of the reaction are cycled along conserved trajectories between hot and cold regions, establishing a chain reaction. In a chain reaction, a substrate gives rise to more than one product which is again a substrate of the reaction. Without restricting boundary conditions, the product amount $c(t)$ will grow exponentially over time t, starting from an initial substrate amount c_0. The speed is described by a doubling time τ:

$$c(t) = c_0 2^{t/\tau}. \quad (1)$$

We consider a DNA replicating chain reaction where the product yields twice the substrate after a short time at elevated temperature. The doubling time thus equals the time between temperature peaks. Convection can realize such temperature oscillation when it is laminar; that is, when the velocity of the flow V, the length scale of the flow L, and the kinematic viscosity ν of the fluid yield a Reynolds number $\mathrm{Re} = VL/\nu$ below 1000. For water and temperature gradients of some 10 °C, we estimate laminar flow for chambers smaller than a few centimeters and larger than $\sim 100\ \mu$m given by molecular diffusion. Periodic temperature cycling therefore can be provided for volumes over 6 orders of magnitude.

We realize by laminar flow the DNA replicating polymerase chain reaction (PCR [1–6]) shown in Fig. 1(a). This chain reaction proceeds in three steps: (i) heating a DNA double helix at 95 °C to dissociate the double helix into two single strands (melting), (ii) decreasing the temperature to a temperature within the range of 50–65 °C in the presence of a large excess of two short DNA fragments (primers), complementary to the terminal sequences of the single strands to be amplified. The primers form short double helices at the ends of the single strands (annealing). The annealing temperature depends on length and sequence of the chosen primers. We have chosen 62 °C to make annealing and the following elongation step at comparable temperatures. In the final elongation step (iii), the temperature is raised to 72 °C in the presence of a temperature resistant polymerization enzyme (polymerase) which elongates each of the two short helices to twice generate complementary copies of the DNA strands to be amplified (product). These three steps are performed periodically by changing the temperature. In a well-designed reaction, the product DNA concentration doubles in each cycle.

In our experiment, the laminar convection is induced by heating from an infrared source (75 mW dissipated power, 1480 nm [7]) in the center of a disk (1 mm × 5 mm). The periphery of the chamber is kept at 52 °C. We calculate the laminar flow using finite element methods (FEMLab, Comsol) from Navier-Stokes and the heat transfer equation under thermal expansion, gravity, radiation pressure by absorption, and also including the temperature dependence of viscosity. The temperature distribution and four flow lines are shown in Fig. 1(a). With a size of the convection $L \cong 2$ mm, typical speeds of $V \cong 0.4$ mm/s and a kinematic viscosity of water $\nu \cong 1\ \mathrm{mm}^2/\mathrm{s}$ we find a laminar flow with Reynolds number $\mathrm{Re} \cong 1$.

The temperature along flow lines [Fig. 1(b)] shows periodic temperature cycling with return times ranging from 6 to 14 s depending on the vertical distance z_0 from the lower chamber wall at half the chamber radius. We confirmed the speed of the simulated convection trajectories with fluorescent beads (4 μm, F-8859, Molecular Probes) as they radially oscillate between the hot center and the cold periphery with periods of 10 ± 5 s along stationary trajectories [Fig. 2(a)].

Although the peak temperature decreases markedly with increasing distance z_0, half of the overall chamber volume reaches peak temperatures above 80 °C and return times larger than 10 s. The achieved temperature variation is similar in shape to a classical PCR temperature profile shown [Fig. 1(c)]. However, the convective flow is roughly 20-fold faster. Based on this analysis, we expect that (i) DNA melts in the hot center, is transported by laminar convection to the colder periphery

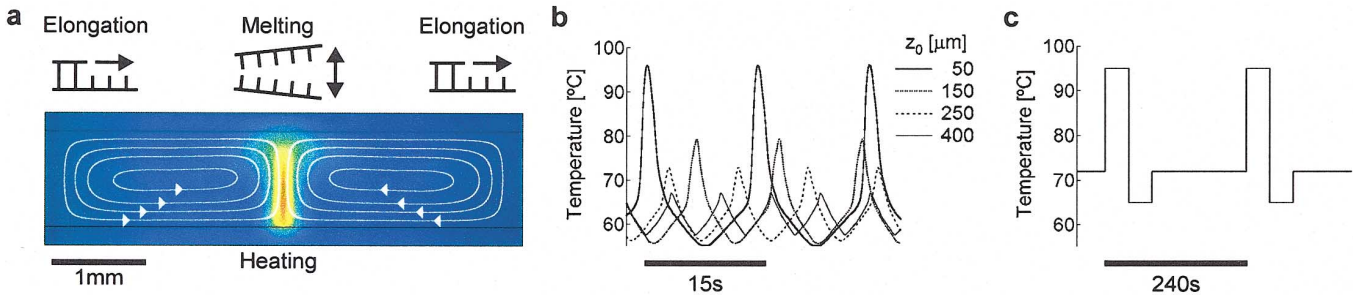

FIG. 1 (color). Convection flow. (a) A flat cylinder chamber (1 × 5 mm, volume 20 μl) is formed between cover slips separated by silicone. The center is heated by focused infrared light. The fluid circulates between the hot center (red) where the DNA melts and the cold periphery (blue) where the DNA elongates. (b) Simulated temperature along the four flow lines in (a) show periodic temperature cycling. With increasing vertical distance z_0 from the lower chamber wall, peak temperatures decrease and cycling periods drop from 14 s down to 6 s, confirmed by beam flow experiments in Fig. 2(a). (c) A standard PCR temperature profile is much slower, but shows a comparable temperature pattern.

where (ii) primers anneal and (iii) are elongated by the polymerase. The cycle starts anew as convection transports the DNA again into the central hot region.

We prepared the reaction as follows. Primers were designed to amplify a 96 base pair piece with melting temperature of 79 °C out of 48 502 base pair long λ-DNA. Primers were 5′-GATGAGTTCGTGTCCGTACAACTGG-3′ and 5′-GGGCAATCAGTTCATCTTTCGTCATGG-3′ with melting temperatures of 61 °C and 62 °C. The reaction mix was prepared according to manufacturer protocols: 5 pmol of each primer, 500 pg of λ-DNA (GibcoBRL), 0.2 × SYBR Green I (S-7563, Molecular Probes) in 25 μl water (degassed by boiling) dissolved one hot start PureTaq-PCR bead (#27-9559-01, Amersham Biosciences).

Convective replication results in a single specific product as revealed by a 4% agarose gel electrophoresis [Fig. 2(b)], comparing 2 μl of XIV-Marker (Roche) against 5 μl of the reaction mix before and after the convection. We start with a 500 pg template λ-DNA (48502 bp) and find about 100 ng of 96 base pair product determined by calibrating against the 100 bp marker spot. The amplification therefore was 100 000 fold within 25 min. The amplification is about 4 times faster than classical PCR thermocyclers, as we will quantify later in the text.

We were able to image the amplification over time [Fig. 2(c)] by specifically staining the double stranded DNA with the intercalating fluorescent marker SYBR-Green I [5] and imaging with a CCD camera (SeniCam, PCO) and a microscope (1.25 ×, Axioscope, Zeiss). Starting from an initial dark image [Fig. 2(c)], the first product is detectable after 8 min and almost homogeneously distributed throughout the chamber. As expected, a central dark spot is visible, corresponding to the region of molten single stranded DNA where SYBR-Green cannot intercalate and is quenched. After the heating is switched off (not shown), the fluorescence image becomes homogeneous due to the final reannealing of the separated product strands in the center.

We studied the gain profile as a function of heat dissipation (Fig. 3). The convection was run for 10 min and analyzed by gel electrophoresis at increasing levels of heating. Starting from zero amplification at 25 mW

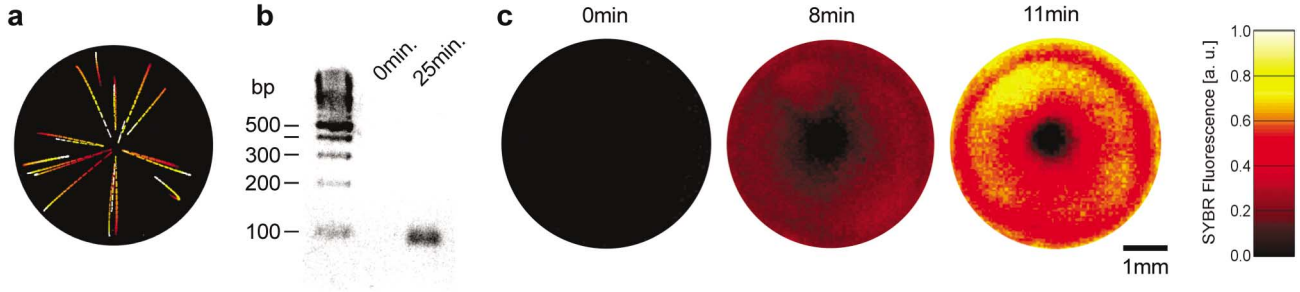

FIG. 2 (color). Convection drives replication of DNA. (a) We image the convection trajectories with fluorescent beads from the top. Both inward and outward flows are in focus. The movement of 13 beads over the time of 5 s is color coded from black to red to yellow to white. Trajectories show periods between 5 and 15 s along the same path as expected for laminar convection. (b) We amplified a 96 base pair piece from λ-DNA and find a 100 000-fold amplification after 25 min. (c) We image the amplification with a fluorescent reporter for double stranded DNA. DNA is visible after 8 min and shows an almost homogeneous distribution. The central dark spot corresponds to single stranded DNA in the heated region.

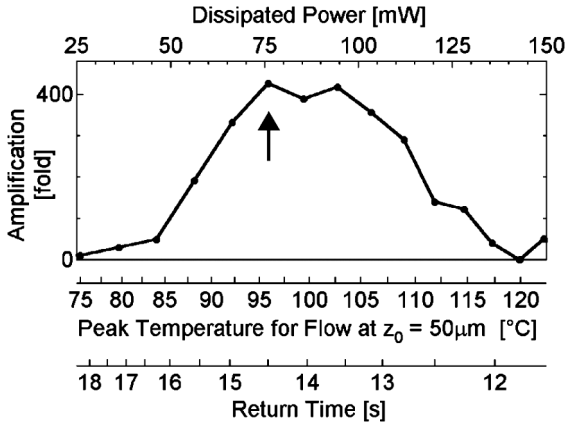

FIG. 3. Amplification as function of heat dissipation. Amplification after 10 min was measured against increasing heat dissipation. As the temperature gradient increases, shown by the peak temperature of a flow line at $z_0 = 50\ \mu$m, the return time of the convection changes only twofold. However the amplification increases from zero to a 400-fold amplification peak at 75–90 mW. Amplification needs to balance between high temperatures in the center to melt DNA and low temperatures in the periphery to avoid denaturation of the polymerase and to allow the primers to anneal.

heat dissipation, amplification reaches 400-fold levels between 75–90 mW. In this range, the peak temperature of the flow line at $z_0 = 50\ \mu$m [Fig. 1(a)] increased from 75 °C to 95–103 °C (Fig. 3). The return time of this flow line decreased only slightly from 18 to 14 s (Fig. 3). The increase in amplification probably is due to the steep melting characteristic of the product in the center of the chamber. Since the heating is localized to the center, we expect other crucial reaction steps such as primer annealing and polymerase elongation to happen in the periphery with similar efficiency.

Further increasing the heat dissipation to 150 mW decreases the amplification gain to zero (Fig. 3). Laminar convection allows overheated water in the center of the chamber without breakdown of the flow. However, an increasing number of gas bubbles form at the periphery of the chamber and slightly restrict the space for convection. We attribute the drop in amplification to inhibited primer annealing as the heating spreads into the periphery of the chamber and to heat-induced denaturation of the polymerase in the center. The convection return time again does not change significantly (14–10.5 s) and should allow enough time for elongation. We therefore explain the amplification gain profile based on changes in temperatures.

Our experiments suggest that laminar convection is probably essential to yield a single product. A communication published very recently showed that nonlaminar Rayleigh-Bénard flow can trigger PCR amplification as well, but results in a smeared band of products after 1.5 h [8]. Furthermore, in our laminar case, flow lines are isolated by diffusion. For example, laminar replication in the upper left corner of the chamber after 8 min [Fig. 2(c)] shows slightly better replication than the neighboring flow lines, an effect which becomes exponentially amplified by the chain reaction.

We show that we indeed have an exponential chain reaction. To do so, we have to monitor the chain reaction before it reaches restricting boundary conditions and runs into saturation. We imaged product DNA concentration over time by recording SYBR fluorescence at high temporal resolution with a photomultiplier in the periphery of the chamber [Fig. 4(a)]. Starting from low fluorescence, the product of the chain reaction rises and reaches a saturation level. We compare five different runs and varied the initial DNA concentration over 5 orders of magnitude. Each run results in a single product [Fig. 4(b)]. With increasing template, the product appeared earlier. The time to reach 10% of the final product was an exponential function of the initial template concentration [Fig. 4(c)]. We fit with Eq. (1) and reveal a doubling time of $\tau = 55$ s. Based on the convective flow we would have expected a doubling time of only about 10–15 s. Reasons for this discrepancy are probably insufficient melting for

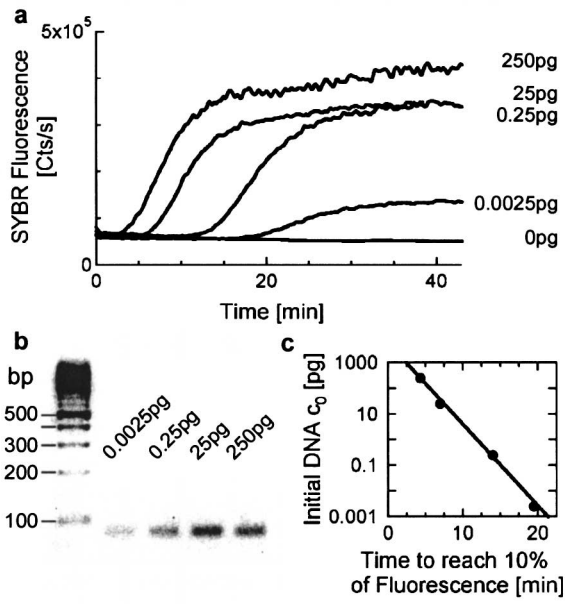

FIG. 4. Exponential amplification. (a) We vary initial template concentrations over 5 orders of magnitude from 250, 25, 0.25, 0.0025 (= 50 000 copies), and 0 pg. We amplified from a 86 base pair single stranded oligonucleotide. SYBR fluorescence is monitored at the periphery of the chamber with a counting photomultiplier. Amplification rises in an exponential growth phase, saturating on a final plateau. No amplification is found without a template. (b) In each case, we find a single product (5 μl on 4% agarose gel versus 2 μl Marker SIV, Roche). (c) The time to 10% of the plateau amplification is an exponential function of initial DNA concentration with a double time of $\tau = 55$ s.

faster flow lines, but incomplete elongation in each cycle is also possible. Both can be optimized. The amplification speed is 4 times faster than classical PCR in standard cyclers [Fig. 1(c)]. Such cyclers heat and cool not only the whole reaction volume, but also the surrounding vessel and its fitting. This leads to high thermal mass which delays heating and cooling. Convective PCR is faster since only the temperature of the liquid is changed and the vessel is kept at a constant temperature gradient. Above results show that real time quantitative PCR can be performed by laminar convection and allows one to measure an unknown initial concentration c_0 by the time it takes to reach a defined product level [6].

Having established PCR in millimeter size chambers suggests that similar reactions could occur in natural environments of comparable scale and temperature gradients, for example, in porous stones and precipitates at submarine hydrothermal vents [9]. Prebiotic scenarios such as the RNA world [10] have suffered from the inability to propagate genetic information stored in RNA sequences following a single cycle of replication [10–13]. Whereas one RNA template can self-replicate with proper raw materials, no natural environment has been proposed to melt the two double stranded RNA molecules for the next replication. Laminar convection has this potential. It can also accommodate temperature cycling on the surface of circulated small particles, extending interface-based molecular evolution [14–17].

To conclude, we have demonstrated a chain reaction in a laminar convection flow. DNA can be exponentially replicated by convection. In millimeter-sized chambers, temperature cycling of laminar thermal convection establishes a fluid-dynamic DNA replicating chain reaction due to periodic heating and cooling. Besides demonstrating a novel approach to high-speed PCR, the results imply new directions for studies of the molecular evolution of life.

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[1] R. K. Saiki *et al.*, Science **230**, 1350–1354 (1985).
[2] R. K. Saiki *et al.*, Science **239**, 487–491 (1988).
[3] H. A. Erlich *et al.*, Science **252**, 1643–1651 (1991).
[4] T. J. White, Trends Biotechnol. **14**, 478–483 (1996).
[5] C. Schneeberger *et al.*, PCR Methods Appl. **4**, 234–238 (1995).
[6] R. Higuchi *et al.*, Bio/Technology **11**, 1026–1030 (1993).
[7] D. Braun and A. Libchaber, Phys. Rev. Lett. **89**, 188103 (2002).
[8] M. Krishnan, V. M. Ugaz, and M. A. Burns, Science **298**, 793 (2002).
[9] N. G. Holm, Originals Life Evol. Biosphere **22**, 1–242 (1992).
[10] *The RNA World*, edited by R. F. Gesteland, T. R. Cech, and J. F. Atkins (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1998), 2nd ed.
[11] H. Kuhn and J. Waser, Angew. Chem., Int. Ed. Engl. **20**, 500–520 (1981).
[12] D. P. Bartel and J. W. Szostak, Science **261**, 1411–1418 (1993).
[13] J. S. Reader and G. F. Joyce, Nature (London) **420**, 841 (2002).
[14] J. P. Ferris *et al.*, Nature (London) **381**, 59–61 (1996).
[15] G. Wächtershäuser, Proc. Natl. Acad. Sci. U.S.A. **87**, 200–204 (1990).
[16] A. Luther, G. Brandsch, and G. von Kiedrowsky, Nature (London) **396**, 245–248 (1998).
[17] W. Martin and M. J. Russell, Philos. Trans. R. Soc. London B **358**, 59–85 (2003).