them — after blood-feeding — to overexpress an introduced gene encoding the antibacterial peptide defensin. Extracts from these mosquitoes killed bacteria *in vitro*. But anti-parasite activity was not reported.

Now, Jacobs-Lorena's group[1] has succeeded in generating a 'strain' of *Anopheles stephensi* mosquitoes that carry an inherited piece of DNA encoding both SM1 and a 'signal' peptide, which helps transport SM1 into the gut cavity. The regulatory region of this artificial gene induces the production of SM1 when needed — after blood-feeding. The authors then allowed transformed and untransformed mosquitoes to feed on the same parasite-infected mouse. In nine experiments the number of transformed mosquitoes carrying oocysts was reduced by 47% on average, and the number of oocysts per mosquito dropped by 80%, relative to untransformed insects. The authors also allowed three groups of transformed mosquitoes to develop for 25 days after feeding, and observed sporozoites at 1.7–19% of control levels. Two of these groups could not transmit the infection to mice. The third showed a 50% reduction in transmission. As the SM1-coding DNA is stably inherited by the mosquitoes' offspring, Jacobs-Lorena and colleagues have shown the feasibility of generating populations of transgenic mosquitoes that have diminished potential to carry the malaria parasite.

This is a proof of principle and as such is a milestone in malaria research. Still, molecular biologists who study mosquitoes fully appreciate the length of the road ahead[11]. Jacobs-Lorena and colleagues[1] used a parasite that causes malaria in rodents, and it remains to be seen whether the SM1 peptide is effective against human parasites carried by other mosquito species. Moreover, to ensure it will be effective in a given location, transformation would probably need to be done in wild populations taken recently from local sites, rather than laboratory strains. There is also no firmly established method for driving transmission-blocking genes through mosquito populations. Too little is known about natural populations and gene flow between mosquito subspecies to allow us to predict the fate of introduced genes. The consensus in the mosquito-research community is strongly against premature field experiments. It is felt that even fully contained field trials must await stringent laboratory experiments and long-term population studies, and that transformed mosquitoes should meet the requirement of a 'significant probability' of reducing malaria prevalence before being released.

The new work[1] is exciting, nonetheless, and represents a new era of malaria-related research. 'Reverse' genetic analysis, in which mutated forms of normal genes are introduced back into an organism to study their function, is becoming routine in *Plasmodium* and no doubt will soon be common in mosquitoes. These techniques will capitalize on the data generated by genome-sequencing projects. In the past few months the raw genome sequence of the most important malarial carrier, *Anopheles gambiae*, became available on public databases[12,13]. Expectations are that by midsummer, the annotated gene content of all three organisms involved in transmitting malaria (humans[14,15], parasites[16,17] and mosquitoes) will be at hand. Thoughtful exploitation of this information should accelerate progress towards new ways of controlling malaria. These will range from new diagnostic tools, effective vaccines that protect people from the parasite and the discovery of new drug targets in the parasite itself, to transmission-blocking vaccines and even mosquitoes that are rendered ineffective as carriers. ■

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Polymer chemistry

Swell gels

Jindřich Kopeček

Linked chains of polymers can form hydrogels, whose properties are attractive for biomedical applications. It seems that the molecular arrangement of the polymer ingredients is central to hydrogel performance.

Hydrogels are three-dimensional networks of polymer chains that swell, but don't dissolve, in water. The formation of hydrogels is an interesting phenomenon, and could one day supplement tools for the design, synthesis and self-assembly of novel biomaterials and drug-delivery systems. On page 424 of this issue, Nowak *et al.*[1] investigate the hydrogels formed by a family of polypeptides that would not usually be expected to adopt such a structure. The hydrogels formed are relatively robust, up to temperatures of around 90 °C, and rearrange rapidly to recover their structure after experiencing stress.

Nowak *et al.* studied diblock copolypeptide amphiphiles — polymers composed of charged (hydrophilic) and hydrophobic blocks of amino-acid residues. In solution, diblock copolymers — which are made of two chemical building blocks, say, A and B (Fig. 1a) — generally form 'micelles'[2]: if block A is soluble in water but block B isn't, in aqueous solution a hydrophobic core of B blocks forms, surrounded by a hydrophilic corona of A blocks (Fig. 1b). But instead of forming micelles, the amphiphiles studied by Nowak *et al.* linked up at low concentrations to form the three-dimensional structure of hydrogels (Fig. 1c). Moreover, this behaviour seems particular to diblock copolymers: random copolymers (Fig. 1a) did not form hydrogels.

The history of hydrogels goes back to the late 1950s when Wichterle and Lím[3] synthesized hydrogels based on copolymers of 2-hydroxyethyl methacrylate with ethylene dimethacrylate — the first biomaterials designed for medical applications. These hydrogels were the original materials used for soft contact lenses, their biocompatibility having been proven during their clinical use as implant materials[4]. The commercial success of soft contact lenses stimulated enormous interest in hydrogels, and eventually led to the development of 'smart' hydrogels that change their properties after exposure to an external stimulus, such as pH, temperature, light or electric field[5–8].

But there are factors that limit the broad application of hydrogels, such as their relatively long response time[9] or a large difference between the time taken to turn 'on' in response to stimuli and the time taken to turn 'off' again. For example, a swollen hydrogel might shrink quickly after exposure to a stimulus, but the re-swelling after reversal of the stimulus would happen much more slowly.

Some of these limitations are consequences of how hydrogels are synthesized. Traditional synthetic pathways, crosslinking copolymerization and crosslinking of polymer precursors, do not permit exact control of chain length, sequence or three-dimen-

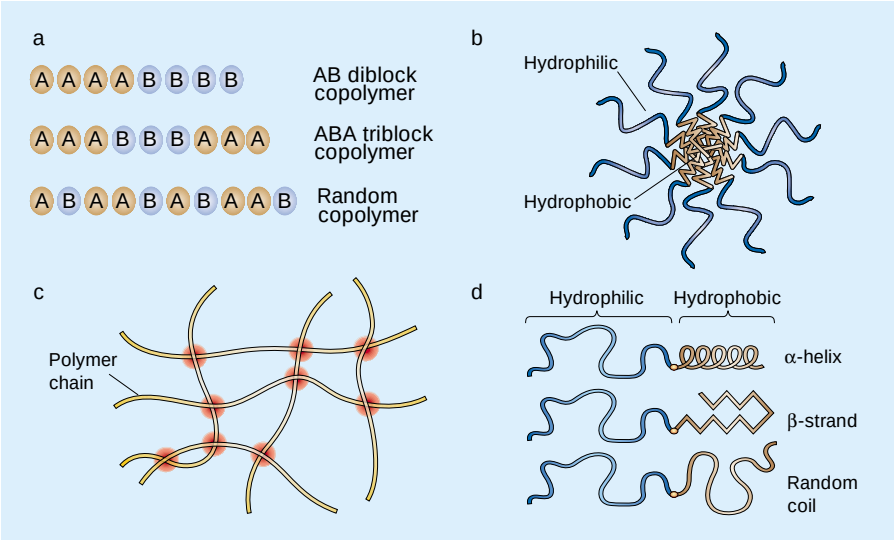

Figure 1 **Copolymers, micelles and hydrogels.** a, **The arrangement of monomer units (A and B) in copolymer chains can take diblock, triblock or random form, and subtly affects the behaviour of the molecules.** b, **Amphiphilic diblock copolymers have a hydrophobic part and a hydrophilic part. In water, the molecules form structures known as micelles, with the hydrophobic blocks of each molecule forming a core surrounded by a corona of hydrophilic blocks.** c, **Hydrogels are three-dimensional structures of crosslinked polymer chains. Nowak *et al.*[1] have shown that some amphiphilic molecules form hydrogels instead of micelles; how easily they do so depends on the shape of the hydrophobic part of the molecule.** d, **According to Nowak *et al.*, molecules whose hydrophobic part is an α-helix are better at forming hydrogels than those with the β-strand conformation or a random coil.**

sional structure. But two new models for the design and synthesis of smart hydrogels have emerged: the synthesis of hydrogels (or their building blocks) by genetic engineering methods and the design of associative building blocks, which self-assemble into hydrogel structures. The combination of these approaches has the potential to be a rational pathway for the design of hydrogels with a rapid on–off response.

Several groups are working on the self-assembly of block copolymers. For example, Tsitsilianis *et al.*[10] have demonstrated hydrogel formation from triblock copolymers (Fig. 1a), and Petka *et al.*[11] have produced triblock copolymers by protein engineering that self-assemble into stimuli-sensitive hydrogels. Diblock copolymers of polyoxyethylene and polyoxybutylene have also been shown to form hydrogel structures, but only at high polymer concentrations[12].

Nowak *et al.*[1] designed and synthesized block copolypeptides with poly-(L-lysine) or poly(L-glutamic acid) as the hydrophilic block and poly(L-leucine), poly(L-valine) or poly(D/L-leucine) as the hydrophobic block. The authors show that these low-molecular-weight diblock copolymers associate into hydrogels at very low polymer concentrations. In particular, the hydrogels maintain their mechanical strength at high temperatures and recover (rearrange) rapidly after stress.

The unique properties of these hydrogels depend on their structural parameters. Nowak *et al.* show that the shape of the polymer chains is an important factor in the hydrogel self-assembly — or gelation — process. They found that α-helical segments are better gelators than β-strands, which in turn are better than random coils (Fig. 1d). Earlier studies of superabsorbing hydrogels[13] have shown that the greater the porosity of a hydrogel, the faster it swells. Nowak *et al.* believe that the fast response times of their hydrogels are also tied in with porosity, as the diblock copolymers were found to separate into a gel matrix and polymer-free liquid domains. They also think that the relatively low molecular weight and narrow molecular-weight distribution of copolymers contribute to the rapid rearrangement of polymer chains and fast recovery of hydrogel structures after stimulus-driven dissociation of polymer chains.

The porosity and fast response of these hydrogels may prove useful in biomedical applications, particularly for drug delivery: molecules, even large molecules such as proteins and DNA, could be loaded into the hydrogel structure to be released as the hydrogel responds to a physiological trigger. Moreover, the degradability of amino-acid-based hydrogel structures *in vivo* is attractive for biomedical applications. Other applications might include the formation of scaffolds for trapping and growing cells for tissue regeneration. One could even imagine that the self-assembly properties of the copolymers studied by Nowak *et al.* could be exploited to develop these materials into sensor/actuator systems, such as controllable membrane-separation systems and electronically controlled drug-delivery systems[12].

100 YEARS AGO

Sun-pillar(?) **Miss Herschel (a careful observer) has just called me out to see one. At 7.10 p.m. she saw the sun above a bank of clouds, in a somewhat hazy sky, but no clouds above it for a space of some 5 degrees. Above that was a light-fringed belt of clouds of great depth. From the sun a parallel-sided pillar of light, just like the reflection of the sun in a slightly rippled sea, stood upright into, and stopped at, the light-fringe; it was not so bright as the reflection spoken of would be, but markedly brighter than the background sky; colour yellow. Miss Herschel had to bicycle home three-quarters of a mile uphill to call me, and it was fading before she reached home. I was prompt, but too late (7.25) to get a good view.** **W. J. Herschel**

From *Nature* 22 May 1902.

50 YEARS AGO

The issue of *L'Astronomie* for December 1951 contains an address by M. Jean Cabannes, president of the Société Astronomique de France, delivered at a meeting on November 18, the title being "Le Ciel Nocturne". In this address an excellent account is given of the night sky, with eight illustrations, most of which are of spectra of the night sky under various conditions: in the visible part of the spectrum, 4000–4900 A.; in the ultra-violet; and in the infra-red, 8300–11000 A., including two of the OH-bands, and others. In spite of our knowledge of the actual atoms and molecules which cause the luminosity in the upper atmosphere, it is admitted that many problems of the phenomenon still remain unresolved. If the luminous layer is thin, what is its height, and if thick, how are the luminous centres distributed in it? If the atmosphere were homogeneous, there would not be much difficulty in determining these altitudes; but as it is heterogeneous, difficulties arise from the necessity of making a long series of measurements to give an acceptable interpretation to the mean. M. Cabannes discusses the possibility of deriving results from rockets equipped with photometers specially adapted for such observations; although the problems are still far from solved, nevertheless the number of astronomers, physicists and chemists who are devoting their attention to the subject is continually increasing, and the successes hitherto obtained give great hopes for the future.

From *Nature* 24 May 1952.

Although these are long-term perspectives, the work of Nowak *et al.*[1] has enhanced our understanding of block copolymers and their assembly into hydrogels. The new information on the relationship between the structure of polypeptide amphiphiles and their properties as hydrogels could one day form a useful basis for the design of new smart biomaterials. ■

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Circadian rhythms

A gut feeling for time

Michael H. Hastings

Many body functions keep a daily rhythm, maintained by a central clock in the brain. But how does the clock communicate with the rest of the body? The small protein prokineticin 2 looks well placed to be the messenger.

Have you ever wondered how you can wake up automatically before the alarm clock rings? It is because your body's neural and physiological preparations for wakefulness are not just a response to the world outside, but are strictly controlled by an internal clock — a region in the hypothalamus of the brain called the suprachiasmatic nucleus (SCN)[1]. Neurons in the SCN have the remarkable ability to autonomously generate a cycle of electrical activity with a period very close to 24 hours. But how might this relatively tiny population of some 10,000 neurons organize the daily (circadian) rhythms of the whole body and keep us in tune with the world? On page 405 of this issue Cheng and colleagues[2] provide a possible answer.

A compelling picture of the core SCN clockwork has been established by molecular genetics. In essence, the canonical clock genes *Period* and *Cryptochrome* make up a self-sustaining circadian oscillator[1], in which the critical mechanism is delayed negative feedback. These genes are switched on by the proteins Clock and Bmal, and are periodically switched off by a complex of their own encoded proteins, Per and Cry. So gene turn-off inevitably follows gene turn-on, in an inexorable daily loop, and mutations that affect the loop's stability are tightly linked to inherited human sleep disorders[3].

A complementary molecular-genetics approach to understanding sleep comes from the study of the gene encoding the hypocretin/orexin peptides. Mutations in this gene, or in that for the receptor protein that enables neurons to detect the peptides, are linked to the sleep disorder narcolepsy[4], in which people uncontrollably fall into brief periods of deep sleep. The cells that make hypocretins/orexins lie in the dorsal hypothalamus and have a powerful excitatory effect on neural systems that sustain wakefulness. But what sits between the oscillatory molecular cycle of the SCN and the neural machinery that directly causes sleep and wakefulness? In other words, what is the messenger of circadian time?

SCN neurons 'fire' in a circadian pattern, suggesting that they signal time by means of conventional electrochemical communication with other neurons. Yet their physical connections with other neurons are sparse and principally local. In fact, intracerebral transplant studies have shown that physical contact between SCN and target neurons is not necessary for them to communicate[5]. Rather, some molecule, presumably secreted from the SCN under the influence of electrical firing, confers circadian control of sleep and wakefulness.

Cheng *et al.*[2] now reveal that a small protein, prokineticin 2, may well be time's messenger. Consisting of 81 amino acids and identified previously as a regulator of gastrointestinal movements[6], this protein fulfils many of the criteria expected of the missing temporal link. It shows high-amplitude, rhythmic expression in the SCN in mice, conferred by regulatory sequences within its encoding gene that are a direct target for positive and negative components of the core clockwork (Clock, Bmal, Per and Cry). It is also downregulated in mice with genetically defunct circadian clocks. As with the *Period* gene, the expression of prokineticin 2 in the SCN is activated by illumination of the retina. So, following a change in the timing of lights on and lights off, the pattern of expression of prokineticin 2 shifts, and so does the activity/rest cycle of mice. Finally, secreted prokineticin 2 is resistant to degradation by protein-cleaving enzymes, making it ideal for long-range signalling in space and time.

How does prokineticin 2 affect behaviour? To find out, Cheng *et al.* injected the protein into the brains of rats. They found that clock time *per se* was not affected, but that the animals became much less active during circadian night — their usual active time — indicating that prokineticin 2 suppresses wakefulness. So prokineticin 2 is not a core clock component, but it does appear to communicate clock time to other brain regions. When functioning normally, the clock would ensure that prokineticin 2 levels peak during circadian daytime, and prokineticin 2 would presumably suppress the wakefulness of rats at that time.

Intriguingly, during the circadian daytime following the nocturnal injection of prokineticin 2 there was a spontaneous rebound, and the rats were active at a time when they should be asleep[2]. This may reflect the fact that the targets of prokineticin 2 in the brain became 'desensitized' during the night because of the excess injected protein, and so failed to respond to the normal protein the next day. It is clearly important to identify these targets, and this is the final piece of news. The receptor protein that detects prokineticin 2 is expressed in brain regions that constitute a roll call of neuroanatomically defined SCN targets, including the dorsal hypothalamus with its hypocretin-releasing cells. Through these targets, SCN-derived prokineticin 2 can signal circadian daytime to several downstream systems, coordinating circadian behaviour and physiology.

The receptor is also expressed abundantly in the SCN, intimating that it may have other roles. Indeed, Cheng *et al.* show that prokineticin 2 can act through its receptor to enhance the Clock–Bmal-driven expression of its own gene. Should this occur within the SCN, it would generate an auto-amplification loop that might explain the massive daytime peak of prokineticin 2 expression, broadcasting an unequivocal midday chime across the hypothalamus and beyond. The mechanism of auto-amplification is unknown, but the activity of Clock–Bmal complexes can be regulated by physiological changes in redox potential[7]. So one possibility is that prokineticin amplifies its own production by modifying metabolism in the SCN.

How might these experimental findings from rodents[2] relate to people? In species that are active during the day, such as humans, the SCN clock mechanism cycles in phase with that of nocturnal rodents[8]: expression of *Period* is high during circadian day in both (but equates to wakefulness in diurnal species, compared with inactivity and sleep in nocturnal rodents). The genetic