the seemingly abrupt appearance in the early Early Cretaceous (Neocomian) of sustained powered flight and endothermic physiology in the first habitually arboreal birds.

5) *Reassessment of early avian phylogeny and ecology*. The advanced flight apparatus and opposable hallux in *Sinornis* and the Spanish bird (*6*) suggest that sustained powered flight and perching capability are primitive for Ornithurae and can no longer be used to unite Ichthyornithiformes and Neornithes to the exclusion of Hesperornithiformes (*18*). These synapomorphies must have been reduced or lost during the evolution of diving habits in Hesperornithiformes. The interrelationships among Late Cretaceous birds are correspondingly less secure, although several additional features maintain a close relationship between Ichthyornithiformes and Neornithes (Fig. 5). Nearly all Mesozoic birds known from reasonably complete remains have been discovered in quiet near-shore marine or marginal lagoon sediments, and this taphonomic bias has colored our view of early avian evolution. The discovery of *Sinornis* in freshwater lake deposits highlights the important, yet largely unknown, role that inland wooded habitats must have played in the early evolution of birds.

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# Competition, Cooperation, and Mutation: Improving a Synthetic Replicator by Light Irradiation


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**Replication and mutation are necessary elements of evolution, and some properties of self-replicating molecules (replicators) can be explored with synthetic structures. Selection and evolution at the molecular level require systems capable of competition and inheritable change. These phenomena have now been observed with synthetic molecules. Two such molecules were prepared having sufficient structural similarity that they catalyzed each other's formation as well as their own. One of the replicators bears a photochemically active function that is cleaved on irradiation. The resulting species is more effective at replication than the original and rapidly takes over the system's resources.**


SELF-REPLICATING MOLECULES CAN be synthesized by covalent linkage of two complementary subunits to give a self-complementary structure (*1*). Complementarity in this context refers to sizes, shapes, and the weak intermolecular forces involved in molecular recognition between the two subunits. Behavior such as autocatalysis and sigmoidal product growth can be expressed by these synthetic replicators as well as by nucleic acid derivatives (*2–5*). For the system to evolve, replicators are expected to make "mistakes," or respond to environmental stresses that favor new and more (or less) competitive species (*6*). Accordingly, we have synthesized structures capable of cooperation and mutation and report here their properties.


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Coupling of the imide ester **1** (Eq. 1) with amines bearing adenine nuclei **2** in $CHCl_3$ yields the respective amides **3** (7). The self-complementarity of these products leads to their extensive dimerization through hydrogen bonding to **4** and is the key to their replicative behavior (*1*).

All three products are replicators: They catalyze their own formation. Specifically, adding 20% of a product to its respective reaction mixture enhances the initial coupling rate by 60% for **3a** and by ~30% for **3b** and **3c** (Figs. 1 and 2). The autocatalysis results from the template effects that gather the two reacting components on the product surface as suggested in Eq. 2. The unsubstituted **3a** can replicate both through Hoogsteen base pairing as shown in **5** and through Watson-Crick pairing as shown in **6**. The urethane-protected **3b** and **3c** are disadvantaged in this respect; the nitrogen substituent hinders base pairing in the Wat-

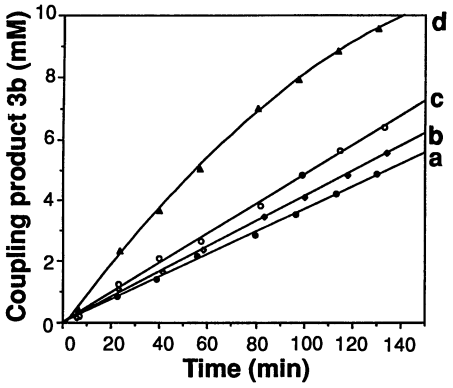



**Fig. 1.** Product **3b** appearance as a function of time for the reaction of **2b** + **1** at 50 mM each in $CHCl_3$: (a) no additive; (b) 20% **3c** added; (c) 20% **3b** added; and (d) 20% **3a** added.

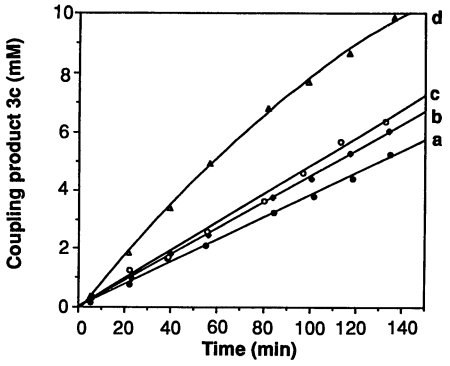



**Fig. 2.** Product **3c** appearance as a function of time for the reaction of **2c** + **1** at 50 mM each in $CHCl_3$: (a) no additive; (b) 20% **3b** added; (c) 20% **3c** added; and (d) 20% **3a** added.

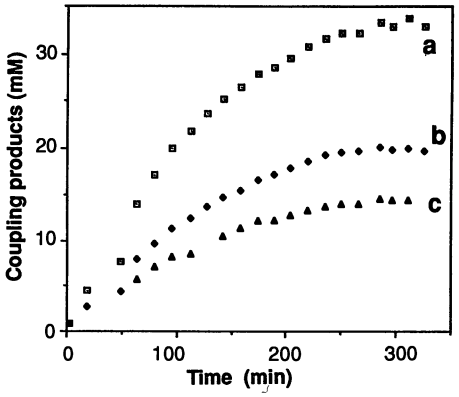
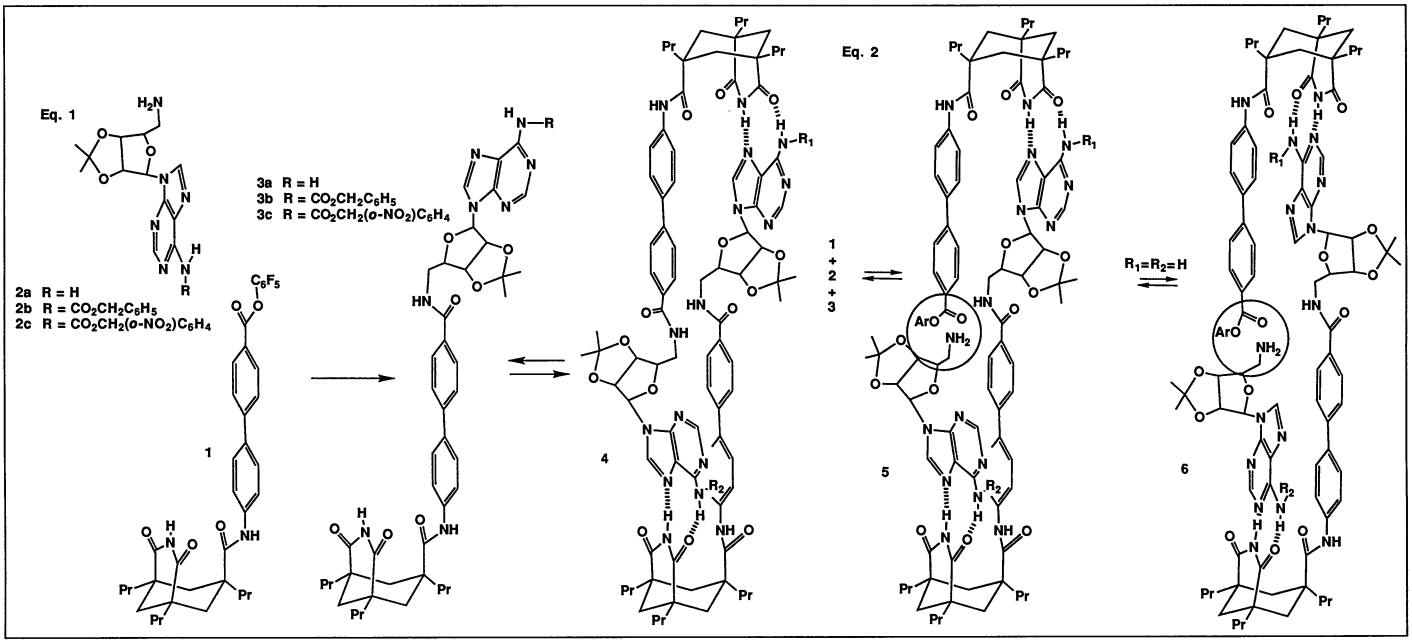



**Fig. 3.** Product appearance as a function of time for the reaction of **2a** + **2b** + **2c** + **1** (42 mM each) in $CHCl_3$: (a) **3a**; (b) **3b**; and (c) **3c**.

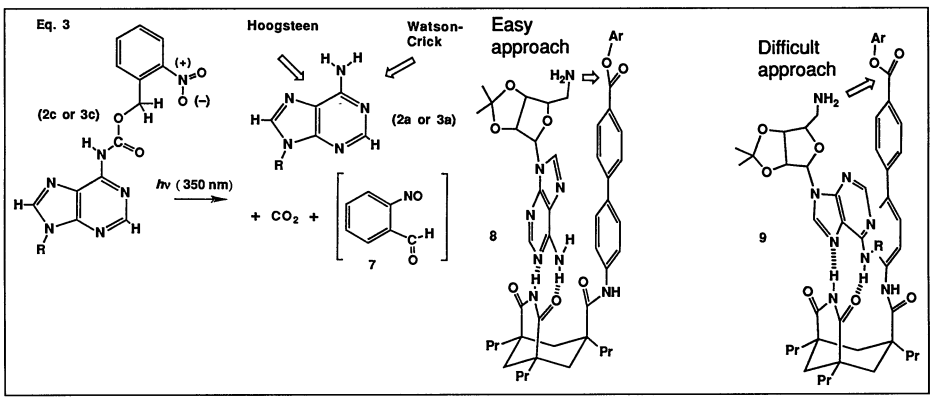



Equations 1 and 2.

son-Crick modes (*8*) and limits it largely to the Hoogsteen modes shown in **5**.

The products do make mistakes: They catalyze the formation of the other replicators. For example, the presence of 20% **3b** during the coupling of **1** with **2c** increases the rate of appearance of **3c** by 18% and the presence of 20% **3c** during the coupling of **1** with **2b** increases the rate of appearance of **3b** by 10%. The cooperative and reciprocal behavior is understandable because the structural similarity of **3b** and **3c** is high; the $NO_2$ versus H difference between the two molecules is at a site remote from their recognition surfaces. In chemical terms, the structures show low selectivity. As a result, the heterogeneous (mixed) termolecular species **5** or **6** ($R_1 \neq R_2$, Eq. 2) and product dimers **4** ($R_1 \neq R_2$, Eq. 1) are as likely to form as the homogeneous ones ($R_1 = R_2$).

The nitrated derivatives are irreversibly mutated by light. Compounds **2c** and **3c** bear photolabile blocking groups (*9*) that can be removed by irradiation at 350 nm (Eq. 3). For example, in $CDCl_3$ a solution of **3c** in a cuvette irradiated for 30 min (Rayonet reactor) is cleanly converted to **3a**; likewise **2c** can be converted to **2a** (*10*). The resulting deblocked system is a more efficient replicator: Direct competition of the three amines **2a**, **2b**, and **2c** for a limited




Equation 3 and structures **8** and **9**.

quantity of the ester **1** results in rapid formation of the efficient replicator **3a** as the dominant product (Fig. 3).

The superiority of **3a** as a replicator is due to its ability to base pair in both senses (**5** and **6**, $R_1 = R_2 = H$) or even a combination of the two. In addition, rapid initial reaction of **2a** with the ester **1** can take place through the Watson-Crick base pair **8** where aryl stacking interactions (*11*) position the reacting functions near each other. In contrast, initial reaction of **2b** or **2c** occurs through **9** where the functions are farther apart. Despite its efficiency, the mutant **3a** is not selfish; it provides effective catalysis for the formation of its competitors, **3b** and **3c**. The presence of 20% **3a** enhances the coupling rates of either of these more than twofold (Figs. 1 and 2).

In the present case adenine-imide base pairing in $CHCl_3$ provides the molecular recognition that leads to self-replication. Other weak intermolecular forces between other host-guest pairs in other solvents could also be used. For example, the solvophobic forces involved in cyclophane-arene complexation in water (*12*) could give rise to a synthetic replicator by way of a covalent linkage between host and guest. The variety of suitable recognition vehicles is vast. Replicating molecules are at the boundary of chemistry with biology, and such synthetic structures can be used to further model evolution at the molecular level.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# Gating of the Cardiac $Ca^{2+}$ Release Channel: The Role of $Na^+$ Current and $Na^+$-$Ca^{2+}$ Exchange


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**In cardiac myocytes, calcium influx through the calcium channel is the primary pathway for triggering calcium release. Recently it has been suggested that the calcium-induced calcium release mechanism can also be activated indirectly by the sodium current, which elevates the sodium concentration under the cell membrane, thereby favoring the entry of "trigger" calcium via the sodium-calcium exchanger. To test this hypothesis, sodium current was suppressed by reducing the external sodium concentration or applying tetrodotoxin. At potentials positive to −30 millivolts, calcium release was unaffected. A small calcium release at more negative potentials could be attributed to partial activation of calcium channels, because it was unaltered by replacement of sodium with lithium and was blocked by cadmium. Thus, sodium influx or its accumulation does not initiate calcium release. In addition, sodium-calcium exchange–related calcium release at potentials positive to +80 millivolts has slower kinetics than calcium channel–induced release. Therefore, only the calcium channel gates the fast release of calcium from the sarcoplasmic reticulum in the range of the action potential.**


IN MAMMALIAN CARDIAC MYOCYTES, the release of $Ca^{2+}$ from the sarcoplasmic reticulum (SR) is controlled by $Ca^{2+}$ influx through the $Ca^{2+}$ channel (*1–3*) by $Ca^{2+}$-induced $Ca^{2+}$ release (*4*). This mechanism is specific to $Ca^{2+}$ because $Ca^{2+}$ can be released by rapid elevation of $Ca^{2+}$ in skinned (*4*) or intact cardiac myocytes (*5*), and neither $Na^+$ nor $Ba^{2+}$ can substitute for $Ca^{2+}$ in the release process when they are the charge carrier through the $Ca^{2+}$ channel (*2*). As an extension of this scheme, it has been reported that the $Na^+$ current can also trigger $Ca^{2+}$ release (*6*) through subsarcolemmal accumulation of $Na^+$ in quantities sufficient to reverse the $Na^+$-$Ca^{2+}$ exchanger and allow $Ca^{2+}$ to enter the cell and trigger $Ca^{2+}$ release (*6, 7*). This could provide a theoretical basis for a beat-to-beat regulation of $Ca^{2+}$ release and contraction by the $Na^+$ current via the $Na^+$-$Ca^{2+}$ exchanger. In this report, we examine the role of the $Na^+$ channel and $Na^+$ accumulation in the subsarcolemmal space in $Ca^{2+}$ release.

Rat ventricular myocytes were enzymatically isolated (*8*) and whole-cell voltage-clamped (*9*). Intracellular fura-2 (120 to 200 μM) was used to monitor the intracellular $Ca^{2+}$ activity. Fura-2 was excited at 335 and 410 nm, and intracellular $Ca^{2+}$ activity was determined from the ratio of the two fluorescence intensities, measured at 520 nm (*3*). The external solution bathing the experimental cell was exchanged rapidly (20 to 100 ms) for short periods (usually 1 to 5 s) with a concentration-clamp system, allowing minimal steady-state alteration of the cytosolic $Na^+$ and $Ca^{2+}$ concentrations.

To examine the role of the $Na^+$ current ($I_{Na}$) in the release of $Ca^{2+}$, we suppressed or abolished $I_{Na}$ by rapidly reducing extracellular $Na^+$ from 142 to 10 mM (for about 2 s) repeatedly at different potentials and analyzed the voltage dependence of intracellular $Ca^{2+}$ transients in myocytes with an intracellular $Na^+$ concentration of 10 mM (Fig. 1). We chose 10 mM external $Na^+$ to set the equilibrium potential of $Na^+$ ($E_{Na}$) at about 0 mV during the experimental run. In a control solution (142 mM $Na^+$), depolarization of the myocyte from −80 to 0 mV activated both the $Na^+$ and $Ca^{2+}$ currents and a maximal $Ca^{2+}$ release. Reduction of the $Na^+$ concentration to 10 mM 0.5 to 1 s before the depolarization of the cell to 0 mV ($E_{Na}$ in test solution) completely suppressed $I_{Na}$ but had no effect on the rate or magnitude of $Ca^{2+}$ release (Fig. 1, A and C). On the other hand, the smaller $Ca^{2+}$ release in the control solution, triggered by depolarization of the myocyte to −50 mV, was abolished by the reduction of the $Na^+$ concentration to 10 mM (Fig. 1, A and C).

The effects of rapid reduction of $Na^+$ were also examined at other membrane potentials, ranging from −50 to +60 mV (Fig. 1, B and D). Despite considerable suppression of the $Na^+$ current, there was no significant difference in $Ca^{2+}$ transients in high- and low-concentration $Na^+$ solutions except at potentials between −50 and −30 mV, suggesting a very limited range of potential for the $Na^+$ current–induced effect. The results were highly reproducible ($n$ = 7 cells) with only minor variations in the degree of suppression of the inward current


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