When Engineers Take Hold of Life: Synthetic Biology

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Scientists today are offering two entirely different visions of living beings. On the one hand, researchers are discovering the fluid, contextual nature of cellular and molecular processes in the organism from countless different angles, with considerable excitement. On the other hand—and with equal excitement—proponents of a relatively new discipline called "synthetic biology" are pursuing the idea that microorganisms, plants, animals, and human beings are machine-like systems consisting of context-independent parts. Synthetic biologists speak of "rationally designing," or reengineering, the organism to carry out functions that they and their funders deem worthwhile.

The fluid and contextual view of life is borne out by countless biological studies. For that reason we could wonder whether synthetic biology's focus on independent parts and its machine view of the organism—a view so little grounded in the biological reality of life—warrants serious consideration. But synthetic biology is propelled forward by highly intelligent and driven engineers and scientists and is funded and supported by large government grants and by venture capitalists who are led to envision myriad products coming down the pike. To be sure, we need to recognize that as a young discipline trying to sell itself to academia, businesses, and funders, synthetic biology can generate enthusiasm that is more or less detached from reality. But it is also true that one-sided and misguided ideas can have tremendous negative impact on the world. They warrant, therefore, careful consideration—and not only after the fact.

We can only hope that organisms themselves will be given due attention and that the shape of the future will not be determined by the free-floating fancies of grant-seeking, innovation-driven scientists and engineers. In the spirit of that hope, I begin with a brief look at what it means to be an organism.

A Power to Grow, Heal, and Adapt

Every healthy human being and animal has the remarkable capacity to heal wounds. When we are injured—cut, bitten, or burned—our body immediately responds. If the wound is not too massive, the blood clots, and a scab and new tissue, including blood vessels, begin to form. Within days or weeks, the healing process, which perhaps also results in the formation of scar tissue, is complete.

When biologists and medical scientists began looking into the details of wound healing at the cellular and molecular levels, they had cause to be amazed at, if not overwhelmed by, the complexity of all the relevant processes. And the more they have discovered, the more it has become clear that there is no "set" of processes, no defined "mechanism" of action in wound healing.

Take, for instance, platelets. As Leslie writes (2010):

> Thirty years ago, researchers were convinced that they had platelets pegged. Every milliliter of our blood, the thinking went, harbors hundreds of millions of these cell fragments for just one reason: to save us from bleeding to death. If we suffer a cut or other injury, platelets swarm into action, forming a plug that seals the wound.

As we now know, "in the absence of hemorrhage, platelets are not essential to wound healing" (Singer & Clark 1999). Moreover, platelets have many functions beyond their contribution to blood clot formation (Leslie 2010; Boyanova et al. 2011; Ware et al. 2013). They produce growth factors that promote healing and substances that help in the re-formation of damaged tissues. They influence the inflammatory response of the body to a wound and its innate immune response in a variety of ways. There are over 5,000 platelet proteins, and although platelets have no nucleus (and are in this sense "cell fragments") researchers have discovered that they do "contain a pool of mRNA which can be spliced and translated in a signal dependent manner" (Boyanova et al. 2011; see also Denis et al. 2005). What this means is that, depending on the substances platelets encounter in the wound environment, they form specific proteins that are effective in that particular situation. Since no two wounds are alike, the healing process varies according to the specific circumstances.

Another example. Connective tissue growth factor (CTGF) was so named because it was initially discovered as a substance that influences the growth of fibroblasts—cells that form connective tissue (Moussad & Brigstock 2000). Later it was shown to be involved in wound healing and the generation of new blood vessels. Over time, many

more functions were discovered (Moussad & Brigstock 2000; Cicha & Goppelt-Struebe 2009). CTGF was found to enhance the growth of other types of cells, but also, under certain circumstances, to have negative effects on cell growth. Depending on the situation, during wound healing it can stimulate the generation of blood vessels, inhibit the growth of new blood vessels, or not be involved in blood vessel formation at all (Cicha & Goppelt-Struebe 2009). It becomes clear that the production and action of CTGF is "a function of the diverse environmental cues to which a cell is exposed at any point in time" (Moussad & Brigstock 2000).

It has become increasingly—we might also say, glaringly—clear that every cell type or molecule is much more multifunctional than originally thought. If researchers study, say, platelets in a particular experimental context, then they may get a fairly defined picture of what they might call "platelet function." But they should call it "platelet function under such-and-such circumstances." When other research groups study different kinds of wounds or inflammatory responses, the functions of the platelets are seen to diversify, depending on the situation.

Clearly, a specific cell type or molecule cannot do everything; it has a limited range of possibilities, but this range is fluid and not predetermined. This is what the research shows for virtually every cell type and molecule in the body. Since, however, cell and molecular biologists are so specialized today and each research group typically focuses on one particular molecule in one type of organism from one limited perspective, the fluidity of the processes becomes apparent only when scientists step back from their own work and review the broader research in their field.*

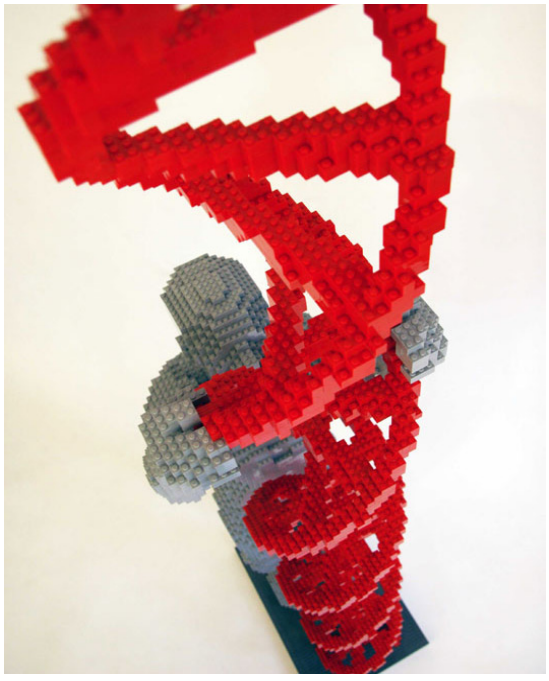
There is an important implication of this research: there are no specific or fixed pathways, and there is no "mechanism" (Talbott 2014). You simply cannot say that cell type X has function Y or that molecule S has mechanism of action T. What biologists hold in mind as determinate pathways are in fact specific realizations of the adaptive, flexible potential of the organism as it manifests in a particular cellular and molecular context. The reality of the mechanism is that it is the mental framework through which the phenomena are viewed; it is not something physically "in" the organism. To limit ourselves to investigations that look for proximal causal relations ("this molecule elicits that response") means to work within a narrow set of highly controlled conditions. We de-contextualize. That is fine, but we should not then forget what it is that we have done to achieve our results. The clarity gained comes at the cost of a loss of fuller reality, which only begins to show itself when we put our findings back into relation to the results of other experiments and we drop the mechanistic framework. What is determinative for one experiment is not determinative in biological reality.

Synthetic Biology

When we turn to synthetic biology, we come up against a very different way of thinking. This may in part reflect the fact that synthetic biologists often have engineering backgrounds, hold patents, and are involved in bioengineering start-up companies, so that they have a financial interest in their efforts coming to fruition.

How, then, do they tend to view living organisms and the task of synthetic biology? James Collins, a leading practitioner and proponent of synthetic biology, studied physics as an undergraduate, holds a PhD in medical engineering, and currently works at Boston University and Harvard. He writes:

> With a box of Lego[s], you can create a whole range of different structures. Snap together pieces of various colours, shapes and sizes to create a multitude of structures—a house, a boat, a tower—with different functions. In the world of biology, a growing group of scientists is thinking about parts of cells in much the same way. Engineers are using genes and proteins as building blocks to create new kinds of cells and new functions for cells. (Collins 2012)



"Building Bricks of Life," by Nathan Sawaya.
Image courtesy of brickartist.com.

* If biologists were to study and take to heart Goethe's seminal little essay "The Experiment as Mediator of Subject and Object"—written in 1792—they would realize the crucial importance of varying experimental conditions in order to gain a realistic picture of a given phenomenon. See: natureinstitute.org/pub/ic/ic24/ic24_goethe.pdf.

In the minds of synthetic biologists, organisms *are* machines, a point Drew Endy, professor of bioengineering at Stanford, makes in stark terms:

> For engineers, biology is a technology … To an engineer, biological systems are replicating machines that make mistakes during the replication process (that is, biological systems are reproducing machines). (Endy 2005)

And these machines can be improved:

> Synthetic biology is bringing together engineers and biologists to design and build novel biomolecular components, networks and pathways, and to use these constructs to rewire and reprogram organisms. (Khalil & Collins 2010)

The "biological machine" is often compared to a computer, here by Craig Venter, who gained fame as the leader of one of the two groups that first sequenced the human genome:

> The genome can be thought of as the software that encodes the cell's instructions, and the cellular machinery as the hardware that interprets and runs the software. Advances in DNA technology have made it possible for scientists to act as biological "software engineers," programming new biological "operating systems" into cells. (Gibson & Venter 2014)

With the notion of the precisely functioning mechanism as their idol, synthetic biologists look down on traditional genetic engineering: It is an "expensive, unreliable and *ad hoc*" technology (Endy 2005) that "generally requires many years of work and trial-and-error experiments to implement" (Arkin 2008). Synthetic biology wants to be more precise and achieve more predictable and controllable results through the application of strict engineering standards:

> Standards underlie most aspects of the modern world. Railroad gauges, screw threads, internet addresses, 'rebar' for reinforcing concrete, gasoline formulations, units of measure, and so on. In the science of biology, a number of useful standards have already arisen around the 'central dogma' that defines the core operations of most natural biological systems...." (Endy 2005)

The "central dogma" Endy refers to is the now outmoded 1960s hypothesis that all the information needed to form an organism is contained in DNA, and that this information is transferred only in one direction: from DNA to RNA to the proteins (enzymes) that in the end are responsible for building up and maintaining the organism. This idea gave DNA (genes) the central position in the "operating system" of the organism. On the assumption that a gene as a particular sequence of DNA determines the structure and function of a particular protein, you can easily conjure up the notion that the organism is built up out of discrete parts—the thousands of genes in DNA. DNA is viewed as a kind of biological code, as Collins views it:

> The genetic code is like any other language: to be able to write it, you have to learn how to read it and understand it. ... Our DNA was once an uncracked code as well, but over the past century, scientists have slowly learned how to read the genetic code that every living cell contains. They have figured out which genes determine which characteristics of cells and organisms, and how changes to genes can alter these characteristics. (Collins 2012)

It should, on this view, be possible to know these parts, to construct new ones for human aims, and to know exactly what these synthetic parts will do in an organism. That is one of the main goals of synthetic biology.

To achieve their aims, synthetic biologists want to construct "standardized biological parts" that can be put together to make "devices" that, when assembled together, would make a "system" (Endy 2005). "We define a biological part to be a natural nucleic acid sequence that encodes a definable biological function and a standard biological part to be a biological part that has been refined in order to conform to one or more defined technical standards" (Shetty et al. 2008). Such standard biological parts are often called BioBricks and they represent "sequences of DNA with specific function that can be combined together to implement more complex functions" (http://syntheticbiology.org/BioBricks.html). There is a public online registry of thousands of such parts (http://parts.igem.org/).

Synthetic biologists speculate that their technologies will help solve many pressing (and imagined) problems:

> What can synthetic biology do for us? How can moving genes around cells, creating biological circuits, and writing new genetic programs change the world? Many of the major global problems, such as famine, disease and energy shortages, have potential solutions in the world of engineered cells.... If scientists can build genes from scratch, they can create organisms with new traits. They can create bacteria that can clean up oil spills, rice with genes that keep the plant infection-free, or cells that can churn out new materials.... What if we could engineer humans with sonar, like that used by bats, to help us navigate in the dark? What if we had genes that enabled us to get energy from sunlight, like plants do? (Collins 2012)

> Synthetic biology is bringing together engineers and biologists to design and build novel biomolecular components, networks and pathways, and to use these constructs to rewire and reprogram organisms. These re-engineered organisms will change our lives in the coming years, leading to cheaper drugs, "green" means to fuel our cars, and targeted therapies to attack "superbugs" and diseases such as cancer. The *de novo* engineering of genetic circuits, biological modules, and synthetic pathways is beginning to address these critical problems and is being used in related practical applications. (Khalil & Collins, 2010; article's abstract)

Clearly, there is a good deal of self-promotion and hype in these statements. Every new technical innovation will, in the eyes of its inventors and promoters, help "solve" significant world problems. Whether it will actually end up doing so or not, or cause new problems that the next ingenious invention will have to solve, remains a question. What in any case is clear is that synthetic biologists pursue a mission—"redesigning," "reprogramming," "rewiring" life and, in the end, creating artificial life. This mission is driven by the image of the organism as a machine-like entity—a notion that permeates all their language. They aim to make living beings into the machines they imagine. They believe that existing life forms are imperfect and mistake-ridden and warrant improvement.

The Gulf Between Language and Facts

The term "synthetic biology" has caught on in the past decade. While it is relatively easy to formulate the engineering conceptual framework and the theoretical goals, it is another matter to discern whether research that runs under the name synthetic biology actually follows its strict engineering principles (Porcar & Peretó 2012).

For example, a new malaria drug, semi-synthetic artemisinin, is viewed as a product of synthetic biology (Peplow 2013). It is a drug that was developed with genetic engineering techniques, chemical synthesis, and also synthetic versions of DNA using synthetic biology principles and techniques (Paddon & Keasling 2014). However, as Porcar & Peretó (2012) point out, all the steps taken to produce this product hardly satisfy synthetic biology's claim of "predictability, lack of noise, orthogonality [i.e. independent functioning of the parts] and standardization." Nonetheless, in their review article, Paddon and Keasling, co-creators of semi-synthetic artemisinin, resort to engineering "synbio speak." For example, they use the term "chassis organism" when they refer to the host organism employed in the development of the drug. As the chassis of a car serves as the framework on which all the parts are mounted, so the host organism serves as structure upon which the biological parts are mounted.

To take another example, Craig Venter and his colleagues published an article in 2010 called "Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome" (Gibson et al. 2010). The article drew widespread attention, in part because people feared that the Venter team had created an artificial form of life. The team does not say they did. But they do say more than what their results—considered in a dry and not hyped-up fashion—warrant. What they did, briefly, was to chemically synthesize a genome, based on the known genome DNA sequence of the bacterium *Mycoplasma mycoides.* The synthetic genome closely resembled—except for additions such as "watermark" sequences for identification purposes—the bacterial genome; it was, in effect, an edited copy of it. The synthetic genome was then inserted into the cell of a different bacterium—*Mycoplasma capricolum*—and the resulting "hybrid" with the synthetic DNA was able to reproduce.

This was a remarkable technical accomplishment. But were Venter and colleagues the creators of a "bacterial cell" or, as they state in their article, a "synthetic cell"? No. They inserted a synthetic genome into a living cell that provided the context needed for the genome to do anything at all. Clearly, they were overstating their case, and it is disconcerting that the editors of *Science* paid no attention to the misleading claim. Commenting on the research, Mark Bedau—philosopher and editor of the journal, *Artificial Life*—more accurately describes the outcome as a "normal bacterium with a prosthetic genome" (Bedau et al. 2010).

The discrepancy between language and actual facts is of real concern. First, the language suggests that organisms are in fact the mechanistic assemblies (think again of the expression "chassis" for a host organism) that synthetic biologists treat them as. Second, the organisms and experiments are described in an engineering style, so that there appears to be more rigorous engineering at work than is actually the case. Third, the results are over-interpreted and framed to favorably fit the mechanistic mission. A kind of hubris takes root in the mind of synthetic biologists who boldly assert that they hold the key to improving organisms.

Living Beings Do Not Consist of "Independent Parts"

It is an important premise of synthetic biology that a standard part (a gene, for example) defines a clearly circumscribed function so that one could construct a device or

The tadpoles of the desert spadefoot toad (which is actually a frog; *Spea multiplicatus*) develop in small ephemeral ponds in the southwestern U. S. and Mexico. Depending on what they feed on, they develop in drastically different ways (Pfennig 1992; Ledón-Rettig and Pfennig 2011).

When they hatch, all tadpoles have the same basic morphology, but if they begin to feed on shrimp and continue to have shrimp as their main food, they develop rapidly, grow large in size, have large jaw muscles, notched and serrated mouthparts, and a short loosely coiled intestine (right in photo). In contrast, their siblings in the same pond (left in photo) may feed on dead organic matter (detritus) and microorganisms. These siblings develop much more slowly, are smaller, and have small jaw muscles, smooth mouthparts, and long coiled intestines.

Other environmental and maternal influences can affect the development of the carnivorous morph, as it is called, and, remarkably, the carnivorous tadpoles can transform back into the detritus-feeding morph if their food is altered. So the specific way these animals form and live depends largely on the active relation they establish with the environment, which in turn influences the formation and growth of their organs and body.

This is anything but machine-like behavior. Synthetic biologists may want to reflect on such realities of biological life when they imagine—and misconstrue—organisms as machines.

system with a predictable outcome. The parts should not do something that has not been foreordained. For this reason, "for engineering purposes, parts are most suitable when they contribute independently to the whole. This 'independence property' allows one to predict the behaviour of an assembly" (Benner & Sismour 2005). Synthetic biologists often speak of independent modules, and the mutual independence of parts is also called "orthogonality."

The question is, do such independent parts exist in real-life organisms? We saw at the beginning of this article in discussing platelets and connective tissue growth factor that this is certainly not the case. Describing what is known about the platelet-derived growth factor (PDGF), professor of genetics and developmental biology, Bruce Mayer, and his colleagues come to the conclusion that "the activated receptor looks less like a machine and more like a ... probability cloud of an almost infinite number of possible states, each of which may differ in its biological activity" (Mayer et al. 2009).

But what about the sequences of DNA we call genes? The same picture is emerging for DNA as it is for all other substances in the body: all its activity is highly context dependent. Geneticists Emmanouil Dermitzakis and Andrew Clark (2009) remark that "we tend to talk about pathways and processes as if they are discrete compartments of biology. But genes and their products contribute to a network of interactions that differ radically among tissues." Such "discrete compartments"—the ideal "parts" of synthetic biologists—do not in fact exist in organisms.

The scientific literature on the biology of organisms is full of such examples. Based on his reviews of current research in molecular biology, Steve Talbott concludes:

> One reason we cannot explain the organism through the relations between parts, is that those parts tend not to remain the same parts from moment to moment. For example, as most molecular biologists now acknowledge, there is no fixed, easily definable thing we can call a *gene*. Whatever we do designate a gene is so thoroughly bound up with cellular processes as a whole that its identity and function depend on whatever else is happening. The larger context determines what constitutes a significant part, and in what sense, at any particular moment. Where, then, is any sort of definable mechanism? (Talbott 2012; see also Talbott 2014)

When biologists begin reckoning with the dynamic and contextual nature of biological processes, the concept of the gene loses any clear-cut demarcation:

> Genes might be redefined as fuzzy transcription clusters with multiple products. (Mattick et al. 2010)
>
> [A gene is] a statistical model to help interpret and provide concise summarization to potentially noisy experimental data. (Gerstein 2007)

The gene has turned out to be a highly abstract and fuzzy concept precisely because the organism is not a mechanism.

And genes are not what make things happen in the organism. Writing in the journal *Science and Education* with the aim to bring science educators up-to-date about the current concept of genes and DNA, Charbel El-Hani and his colleagues emphasize that "it is not DNA that does things to the cell; rather, it is the cell that does things with DNA. This is, indeed, one of the major conclusions we can take from developments in the debates around the gene concept in the last three decades..." (Meyer et al. 2013). Because of this context dependency, genes should be, in their words, "conceived as emerging as processes at the level of the systems through which DNA sequences are interpreted, involving both the cellular and the supracellular environment. Thus, genes are not found in DNA itself, but built by the cell at a higher systemic level."

The reality of "parts" within organisms is that they are not definable independent entities but rather interconnected and dynamic processes or potentials that respond and change in relation to changing situations. This is hardly the notion of a "standard biological part." At least to a degree, this is recognized by some synthetic biologists, such as Timothy Gardner and Kristy Hawkins, who write: "natural biological parts are often not modular. Small changes from part to part, or the molecular context in which the part is situated, produce oft-times significant variation in the functional behaviors" (Gardner & Hawkins 2013).

The Failure of Synthetic Biology Systems

Given the fact that the synthetic biology framework does not conform with organismic reality, it is not surprising that synthetic biology design experiments have often failed to work. This has not, of course, gone unnoticed by the synthetic biology community. A review article by synthetic biologists Stefano Cardinale and Adam Arkin of the Lawrence Berkeley National Laboratory tries to identify the "causes of failure of synthetic biology systems" since all too often, as they state, "molecular and genetic devices inexplicably fail to function as designed when tested in vivo" (Cardinale & Arkin 2012).

Spanish systems and synthetic biologist Victor de Lorenzo (2014) writes that "synthetic biologists have created a large number of genetic circuits in which transcription factors and promoters are rationally re-connected following a man-made blueprint aimed at programming new-to-nature properties" (see also Khalil & Collins, 2010, for many examples). De Lorenzo points out that "it is now common knowledge that such devices operate for a limited period of time, after which they often succumb to noise and mutations."

For instance, part of the genome of the T7 bacteriophage—a virus that infects bacteria—was reconfigured ("refactored") by scientists (Chan et al. 2005). The modified phage was able to infect bacteria—it was functional in this sense and is cited as an early example of successful synthetic biology. However, "its subsequent evolution in vivo whilst progressing towards recovering the fitness level of the wildtype phage erased 40% of the manmade modifications. In contrast, naturally occurring regulatory circuits are quite robust, and maintain their performance across time and space" (de Lorenzo 2014).

Part of the "problem" of real organisms is that they live in variable environments and can respond meaningfully and in a variety of unpredictable ways to those variations. So one strategy of synthetic biology is to create highly uniform and stable conditions in the environment so that the organism with its new synthetic parts is not subjected to the myriad perturbations in real-world life. Therefore, writes biotechnologist and bioengineer Martin Fussenegger in a sober assessment, "should a species with a programmed synthetic genome one day become useful, it would probably be contained in specific production environments" (Bedau et al. 2014). He's thinking of micro-organisms carrying out specific processes or producing specific products in highly controlled industrial conditions.

Making Machine-Like Organisms?

But the goal of synthetic biology is not only to control the environment, but also to control internal functions of organisms. Therefore the contextual, situation-dependent activity of the organism at all levels presents a major challenge; it is a "barrier to predictability in design" (Cardinale & Arkin 2012).

In a moment of circumspection synthetic biologists Bashor and Collins admit that "engineered biological circuits rarely work as designed. In most cases, the performance of their molecular parts is highly dependent on cellular and sequence context and varies greatly from one system to the next" (Bashor & Collins 2012). What is their response to this challenge? Unfortunately, they do not rethink their approach in light of the reality of organisms. No, the unwieldy nature of organisms needs to be overcome. "Synthetic biology urgently requires strategies to limit such context-dependence."

We should let this sentence sink in. Limiting context dependence means making an organism less of an organism and more machine-like. It means limiting the spontaneity, unpredictability, and flexible responsiveness that are integral to life. So if synthetic biology actually follows its own principles and strives, in its view, to "improve" plants, animals, and human beings, then it will "succeed" to the degree that it limits or eliminates essential characteristics of life.

Given the degree of technical sophistication, zeal, intelligence, and funding that supports synthetic biology, I have little doubt that, left to its own devices, it will forcefully pursue this goal. And since living beings are above all else adaptable, I can imagine that synthetic biologists will find ways to make them accept and adapt to their machine-like assemblies. But I'm even more certain that along the way much will go wrong and there will be many unintended consequences—for the organisms themselves and for the larger environment as well.

What is particularly disturbing about synthetic biology is that we know today that organisms are not machine-like assemblies. So why would we want to implement an inadequate framework? Is the deeper motivation that the engineering mind simply wants to follow its fascination with absolute control and predictability? Shouldn't we consider more thoughtfully what it means when human beings engage in the activity of making other living beings and perhaps ourselves into less-than-living "systems"? Can we do that responsibly? What boundaries can or should be set? Who can set such boundaries?

Whether and how these questions are addressed should not be left up to the community of synthetic biologists and its funders, given their mission-driven zeal and power. These are urgent questions that warrant attention and consideration by a larger community of concerned lay people, environmentalists, scientists who are not proponents of synthetic biology, policy makers, and, yes, synthetic biologists.

Of course, who of us comprehends life and knows all we should know before we act? But there are two different kinds of ignorance. We can study the phenomena of life and realize life's intricacies, its remarkable plasticity, its context-sensitivity, its aliveness. In the process of gaining this knowledge we begin to realize how little we know. This is wise Socratic ignorance—knowing you don't know. It is a kind of ignorance that encourages circumspection and caution in action. But there is also the very different ignorance that Herman Wouk captures when he writes about someone being "too clever to be wise." This ignorance is blinded by its own intelligence, ignores what it does not want to see, and strives to bend reality to fit its mission: man manipulating life in service of the machine idol. This ignorance fosters hubris that tends—because it thinks it knows best—to run roughshod over the intricacies of life.

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