# Explaining the Evolution of Design across the Biological World by Identifying Active Replicators

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## Introduction

There are three key ingredients necessary for a population of organisms to undergo evolution by natural selection. First, there must be variation between individuals in the population (some cheetahs run faster than other cheetahs). Second, there must be differential reproduction between the individuals (the faster cheetahs leave more offspring), and finally, there must be some mechanism of heredity (the offspring of the fast cheetahs are pretty fast themselves). This is Darwin's theory of evolution by natural selection, and it immediately allowed him to explain how the incredible designs we see in organisms came to be, selected for their particular functions over the course of millions of generations. What the theory did not explain, however, was how any of these requirements were achieved in biological systems. Darwin could only speculate about the mechanisms of heredity and variation in a population.

Around the turn of the 20$^{th}$ century, after the work of a monk by the name of Gregor Mendel was rediscovered, biologists began to realize that in many cases, heredity followed certain patterns (*The Mendelian laws of inheritance)* that could be modeled by the independent segregation of units of heredity, the *classic Mendelian gene*. When insights from the new field of population genetics showed that these Mendelian laws of inheritance also modeled the changes that take place in whole populations of organisms, and that these changes agreed with Darwin's theory of evolution by natural selection, the modern evolutionary synthesis was born, the account of evolution that is still widely accepted today. But incredibly, a mechanistic explanation of how and why these Mendelian laws explained the differences in phenotypes in populations was *still* lacking. Finally, in the early 1950s, a series of brilliant experiments confirmed that DNA was the hereditary material and the molecular revolution took off in earnest. Over the course of a couple of short decades, it was discovered that sequences of DNA are transcribed into RNA that are

subsequently synthesized into proteins, the building blocks of life, in every single living cell. The molecular details underlying the replication and expression of the hereditary material had finally been illuminated.

Over the course of the past several months, I have set out on a mission to integrate these mechanistic details of heredity with Darwin's theory of evolution by natural selection. This is no easy task. The molecular details that explain how the hereditary material can produce a complex trait, especially in higher organisms, are incredibly complex; even those with a background in molecular biology find it hard to fathom how variations in the DNA can be differentially selected through the phenotype of the organism, resulting in cumulative change over many generations. My goal here is to develop a framework we can use as a tool to wrap our heads around the complex mechanistic details of heredity in the context of Darwin's theory. We will use this framework to reach a more complete understanding of the incredible designs we observe across the natural world that Darwin could ever have imagined.

The framework I will advocate for is called *replicator selectionism*. This framework is a variation of the gene's eye view of evolution, or gene selectionism, a way of looking at the biological world first articulated by Richard Dawkins in the 1970s. Although the gene's eye view was a brilliant way of explaining cumulative evolutionary change given what was known about molecular biology 40 years ago, I will suggest that in order to account for what we now know about complex gene expression patterns, the gene's eye view must be amended. In section 1, then, I will argue that the replicator/interactor distinction that is at the heart of the gene's eye view of evolution should be maintained, but that we should reject the notion that *genes* are active replicators. For the remainder of the thesis, I will show how robust identification active replicators, using the criteria articulated by Dawkins himself, is the crucial first step in

explaining complex design in both asexually and sexually reproducing organisms. In section 2, we will see that parts of entire chromosomes, plasmids, and transposons are active replicators in asexually reproducing organisms like bacteria. Before moving on to identify the active replicators in sexually reproducing organisms I must justify a philosophical move I make in which the consistent *molecular* effects of active replicators instead of their consistent overall *phenotypic* effects is a requirement of replicators in sexually reproducing organisms. This justification is made in section 3. In section 4, I identify the active replicators in the coding regions of sexually reproducing organisms as domain-coding DNA fragments, and explain how differential selection of domain-coding fragments can account for the appearance of features of organisms through a hierarchy of interactors. Finally, in section 5, I argue that regulatory elements are also important active replicators in sexually reproducing organisms, and help explain how responses to environmental changes and other phenotypic *changes* in sexually reproducing organisms evolved.

## 1. From Gene Selectionism to Replicator Selectionism

One of the natural questions to ask when marveling at features of the biological world, such as an elephant's trunk, is "What entity is the elephant's trunk *good for*?" For the first century following the publication of *On the Origin of Species*, evolutionary biologists assumed that *organisms* were the beneficiaries of these amazing features, a natural assumption to make considering such adaptations increase the fitness of the organism. Organisms were firmly placed at the center of evolutionary theory as the *units of selection*, or the entities upon which natural selection acts in the *long term* (Lloyd 1992). But following the molecular revolution of the 1950s

which uncovered DNA as the basic hereditary material, a powerful new way of looking at the basis of evolutionary change emerged: gene selectionism, or the gene's eye view of evolution.

Gene selectionism holds that individual fragments of DNA called *genes* are the ultimate beneficiaries of evolutionary processes, and the units of selection. This view is composed of two main principles. The first principle is a distinction between *replicators* and *interactors*, two entities which both play essential but very different roles in the evolutionary process; replicators are the entities which are copied each generation, and interactors are the entities which interact with the environment, resulting in the differential copying of the replicators. The second principle is that *genes* are replicators in sexually reproducing organisms. Later, I will argue that the units of selection cannot be genes but are actually *different* fragments of DNA, including protein-domain coding regions and transposable elements. My view thus accepts the first principle of gene selectionism, the replicator/interactor distinction, but rejects the second principle, that genes are replicators. In this section, then, I will evaluate the arguments in favor of these two principles of gene selectionism. First, I will show that the replicator/interactor distinction is the right way to think about evolutionary change across species. I will then argue that genes cannot be the units of selection because genes do not possess the consistent phenotypic power, a necessary feature of active replicators.

### 1.1. Replicators and Interactors

Let's begin by taking a closer look at the distinction between replicators and interactors, and the motivations for making the distinction in the first place. We'll start by examining the features of the replicators, mainly drawing from *The Extended Phenotype* (Dawkins 1982), the classic and most developed defense of the "active germ-line replicator" concept. According to

gene selectionism, one of the requirements for evolution by natural selection is replication of certain structures in an evolutionary system, and it is these replicated structures which are identified as the active replicators in Dawkins's view. So even though the bodies of organisms (the *interactors*) reappear every generation, they are not directly copied and transmitted from parent to offspring. Fragments of DNA, on the other hand, *are* directly copied by the DNA replication machinery and inherited by the next generation, either as a direct copy of a set of DNA molecules from one parent in asexually reproducing organisms or as a mix of copies of DNA from the germ line of two parents in sexually reproducing organisms. Thus, just from the requirement that replicators must be directly copied, it is relatively straightforward to identify DNA as the material stuff active replicators are made of in the vast majority of evolutionary systems[1].

Now, one alternative is to think of active replicators as fundamentally consisting of units of *information* rather than physical entities. In this case, we might say that it is actually the information that is copied every generation (Williams 1992). While it is common among biologists to speak of DNA fragments as *coding for* certain protein molecules under the assumption that there is some sort of information present in the genome, philosophers are in profound disagreement about the explanatory utility of saying that the genome contains information, and if it does, what kinds of information it contains (Griffiths and Stotz 2013). Much of the debate has focused on how much causal specificity of phenotypic structures is contributed by the genetic material and how much is contributed by the environment. For instance, Kenneth Waters (2007) argues that coding sequences are the principle source of

[1] In some rare cases, RNA molecules are copied and transmitted, in which case fragments of RNA qualify as active replicators. For instance, retroviruses infect cells with RNA which hijacks the cellular machinery, inducing many copies of the viral RNA to be produced. In addition, according to the RNA world hypothesis (Higgs and Lehman 2015), free-floating RNA active replicators that could induce their own replication were the origin of life.

informational specificity for all molecular interactions, while Griffiths and Stotz (2013) argue that the environment and epigenetics play such an important role in determining molecular specificity that the genome only contains "Crick information – the causally specific determination of the order of elements in a gene product (179)." This is an important debate to have, because if we can say that genetic replicators are really informational entities then it might be possible to apply the replicator/interactor framework to evolutionary dynamics which are not based in material genetic entities, including cultural evolution. It is also necessary to consider replicators as informational entities of some sort if we want to argue that the replicator/interactor distinction is a necessary, rather than sufficient, condition for evolution by natural selection. However, my goal here is only to explain the origins of the features of real organisms that we observe, and for this purpose I don't believe that it is *necessary* to think of active replicators as informational entities. Instead, we will leave the debate about the informational replicator concept for another time and see that the *effects*, broadly speaking, that DNA fragments have on their corresponding interactors is the only causal chain that must be set up to explain complex adaptation.

So far, we have established that we will consider fragments of DNA as active replicators because DNA is directly copied every generation. More difficult, however, is the identification of which *kinds* of individual DNA fragments qualify as active replicators, and to this end Dawkins introduced several more criteria that active replicators must possess. First, the DNA fragments that qualify as replicators must form potentially immortal *lineages* that persist relatively unchanged through thousands of generations. A lineage is a chain of identical copies of a certain replicator, copied and transmitted generation after generation; in philosophical jargon, the individual copies of a replicator are called *tokens* while the collection of tokens is called a

*type.* The ability for active replicators to form a lineage will become important later as we look at differences between active replicators in asexually and sexually reproducing organisms. A second important feature of replicators in biological systems is that the replicators must have the ability to actively influence the probability that they are copied, hence the term *active* replicator. DNA fragments are clearly active replicators, as the fitness of the organism DNA helps build will ultimately determine the probability that the DNA is passed on to the next generation. Thirdly, active replicators have phenotypic power, or a consistent phenotypic effect on the interactor they help create. I will examine this property in detail later in this section when I argue that genes do not have this required phenotypic power. Finally, it is important to note that true replicators are located in the *germ-line* of sexually reproducing organisms. Only the small population of germ-line cells located in the gonads of sexual reproducing organisms has the possibility of passing on their haploid genome to the next generation. Dawkins labels DNA in somatic cells "dead-end replicators", as these replicators will never have a chance to be represented in next generation, no matter how many times they are copied (they cannot form an immortal *lineage*). When I refer to replicators from this point forward, I will be specifically referring to these "*active germ-line replicators"*.

The second necessary feature of evolution by natural selection according to gene selectionism is the differential survival of organisms, by the interaction of organisms with the environment. *Interactors*, then, are the entities which interact with the environment and cause the differential survival of the replicators that reside in, or produce, the interactors. Now, Richard Dawkins favors the term *vehicle* instead of *interactor* (The Extended Phenotype 83), and the two terms are often used interchangeably. There is a subtle difference between the terms, however, which will become relevant later when I will argue in favor of a hierarchy of interactors.

Vehicles are coherent, distinct units which house a collection of replicators. This term identifies, with few exceptions, whole *organisms* as the entities which interact with the environment. On the other hand, David Hull suggested the substitution of the term vehicle with *interactor* to facilitate an argument that organisms are not the only types of interactors (1980). While Hull claims that there are entities more inclusive than organisms that might function as interactors, such as colonies or ecological communities, my view considers interactors smaller than a single organism, such as individual proteins, as entities which interact with the environment to promote the differential survival of replicators. So although I still think *vehicle* is a useful term, I will favor Hull's interactor over Dawkins's term vehicle here as the entity that interacts with the environment resulting in the differential selection of the active replicators.

With the replicator/interactor distinction clear, let us now examine the powerful arguments that favor this first principle of gene selectionism, identifying replicators as the units of selection. The primary motivation for distinguishing replicators from interactors was first articulated by George Williams in *Adaptation and Natural Selection*, and endorsed by Dawkins in *The Extended Phenotype*. Dawkins and Williams argue that for cumulative natural selection to occur, in which many small changes over many generations lead to complex adaptations, natural selection must act on entities that are directly copied with high fidelity over many generations. This is because any modifications or mutations to entities, which are the basis of evolutionary change, must be copied into the next generation in order for the advantageous modifications to be selected. Organisms and their phenotypes are only temporary manifestations; a modification in the phenotype of an organism, such as the loss of a limb in an insect, is *not* transmitted to subsequent generations because phenotypes are not directly copied. A change in the DNA of the germ line of an organism, on the other hand, *is* transmitted. So while features of both DNA and

features of whole organisms are *heritable* (the features of the offspring simply have a high probability in resembling the features of the parents), there is only *transmission* of genetic structures. Furthermore, there are causal arrows leading from replicators in the paternal generation to replicators of their offspring, and from replicators to phenotype, but *no* causal arrows from phenotype to replicators; DNA causes the copying of DNA through the interactors they build, and changes in the DNA cause changes in phenotype, but changes in phenotype will *not* cause changes in the DNA. This principle, often called Molecular Weismannism, is the extra premise required to explain why lineages of DNA copies, but not lineages of phenotypes, should be regarded as the units of selection (Sterenly and Griffiths 1999). As a result of this principle, any mutation acquired in the genetic structures will be forever passed down in that particular replicator lineage if it is not picked up by the DNA repair machinery. In this view, replicators are granted a special status as the *units of selection*, since modifications to the replicators, but not to the interactors, results in cumulative natural selection over many generations.

Now, although gene selectionism identifies replicators as the units of selection, there is no denying the importance of interactors. Interactors, as we have already seen, play an equally essential but very different role in cumulative natural selection: they interact with the environment to cause the differential survival of replicators, ensuring that the advantageous modifications replicators pick up along the way are more likely to be transmitted. Evolution, then, consists fundamentally of two distinct processes according to gene selectionism: replication and interaction. Distinguishing between the two entities that participate in these different processes clearly shows how transmission of directly copied DNA structures results in cumulative natural selection.

Despite this advantage of adopting the replicator/interactor distinction, there has been no shortage of objections to this framework over the past 30 years calling for major amendments or even the abolition of the replicator concept. Much of the criticism has focused on the special status that proponents of gene selectionism grant to the replicators, and the perceived subordinate importance of environmental and developmental effects in this evolutionary framework. For instance, proponents of developmental systems theory (DST) claim that the entire life cycle of an organism is the entity that replicates, and that the replicator/interactor distinction incorrectly leads us to assume that developmental processes have distinct genetic and environmental components (Griffiths and Gray 1994). Others object to the idea of using strong causal language to describe the powers that replicators have to "produce" or "create" the interactors that act to further the copying of the replicators (Godfrey-Smith 2000). These critiques have been very useful in identifying where gene selectionism has been a bit too eager about using unqualified metaphorical language. Think of Dawkins's oft-quoted passage from *The Selfish Gene* (1976) about replicators manipulating giant, lumbering, robotic organisms by remote control. It is no wonder that the replicator/interactor distinction was often misinterpreted as an argument in favor of genetic determinism, the view that it is exclusively the genetic material that determines the fate of an organism. But rather than abandoning the replicator/interactor distinction or the useful causal language altogether, I suggest that we integrate these criticisms into careful qualifications when articulating the principles behind the replicator/interactor distinction. This involves stressing the environmental influences on development that affect an organism *after* the genetic program sets the initial switches, showing that the interactor is just as important in the evolutionary process as the replicator, and, as Dawkins was careful to do in *The Extended Phenotype*, make sure that the replicator/interactor distinction is not mistaken as genetic

determinism. This way, we can preserve the important explanatory power the distinction has for cumulative natural selection leading to complex adaptation.

### 1.2 Genes are not active replicators

In order for the replicator/interactor distinction to be a truly useful tool for thinking about evolution, it is of course necessary to identify exactly which fragments of DNA qualify as replicators, since at its basis natural selection is about the differential survival of different versions of replicators. It is finally time to acknowledge the word "gene" in gene selectionism: the second principle behind gene selectionism is that *genes* are the replicators in sexually reproducing organisms. This principle is much more controversial than the replicator/interactor distinction, and rightfully so, as I will argue here that genes should not be regarded as replicators in the replicator/interactor framework. It is Richard Dawkins that makes the strongest arguments for why genes should be considered replicators in *The Extended Phenotype*. Thus, I will spell out his line of reasoning here and show why it is wrong.

To show how replicators are genes, Dawkins first precisely describes what characteristics genetic replicators have, before arguing that genes have the same characteristics. So, let us begin by closely examining the characteristics of Dawkins's genetic replicators. Dawkins argues that a genetic replicator must possess a number of fundamental properties. As with any active replicator, genetic replicators must by copied and have the ability to influence the probability that they are copied. This much is true of fragments of just about any size that are located in coding region of the genome, as the protein products that they produce will ultimately determine the success of the interactor in copying the replicators. Similarly, just about any fragment has the requisite fidelity and fecundity required of a replicator. But in order to form a *lineage* that is

subject to cumulative natural selection, the fragment of DNA that qualifies as a replicator must also possess a certain amount of *longevity*, or the ability to be copied *as a single unit* for many generations. In sexually reproducing organisms, this property rules out larger fragments of DNA, because chromosomes are regularly broken apart and rearranged in the process of crossing over during meiosis in each generation. Only by choosing a fragment small enough to avoid this constant reshuffling is it meaningful to speak of replicators that are more or less successful in the evolutionary game, Dawkins correctly argues, since whole chromosomes that are broken apart every generation never have a chance to be differentially selected as whole entities. This property of replicators closely mirrors what is called the *evolutionary gene concept*, developed by George Williams: "in evolutionary theory, a gene could be defined as any hereditary information for which there is a favorable or unfavorable selection bias equal to several or many times its rate of endogenous change" (Adaptation and Natural Selection 25). In fact, Dawkins routinely equated properties of a replicator to the properties of Williams's evolutionary gene before realizing that for the active replicator property to be useful, the DNA fragments that qualify need one more very important property: phenotypic power.

Dawkins and his critics soon recognized that if fidelity, fecundity, and longevity were the only properties needed to identify genetic replicators, then tiny fragments of DNA, including single nucleotides, would qualify as replicators. A single adenine at a particular locus in the coding region of the genome will reappear in the exact same position for many generations (barring any mutations), will be accurately copied, and will influence the probability that it is copied by contributing to the structure of some protein. This absurd reduction of the replicator concept is problematic for a few reasons. First, if single nucleotides act as replicators, the replicator/interactor distinction loses much of its explanatory power, since it become very

difficult to see how *cumulative* natural selection occurs over many generations, modifying replicators little by little, if natural selection is acting on a single entity that can exist in only one of four possible states (Adenine, Thymine, Guanine, or Cytosine). The second issue is even more problematic: if every adenine in the genome is a single replicator, there is no meaningful way of saying that the adenines, in general, are in competition with other nucleotides. The adenines at each locus experience very different selection pressures, so it does not make sense to speak of differential selection between adenines and a modification of the adenine to a guanosine *as a group*. At a single locus, there is of course meaningful competition between the four nucleotides; it might be the case that a G at a certain position is more successful than an A in that position, leading the G replicators to be more successful than the A replicators at that particular locus. So one way to amend the replicator concept might by to say that each nucleotide at each locus is its own replicating entity, and that the adenine at position 139 has no relationship to the adenine at position 140. While it is true that meaningful competition can only take place between two replicators competing for the same locus, treating each nucleotide as a replicator would mean that the human genome contains 3 billion individual replicators. At the very least, this is not a useful way of describing what is going on; since the physical identities of many replicators would be the same (there are 750 million adenines in the genome), the physical location would be the only way to distinguish between the different replicators. This would become quite confusing considering the many insertions and deletions that are part of normal molecular evolution. And, as previously mentioned, it is difficult to see how treating individual nucleotides as replicators can clearly explain cumulative natural selection and complex adaptation. Dawkins wisely took another route to avoid the "selfish nucleotide" reduction, abandoning the evolutionary gene concept and arguing that replicators also must have *phenotypic power*.

The property of phenotypic power means that the replicator asserts some *consistent phenotypic effect* on the world, regardless of the genetic or molecular environment finds itself in. This property effectively rules out individual nucleotides as replicators, since the phenotypic effect of an adenine is completely dependent upon the sequence context in which the nucleotide exists. This amendment to the replicator concept is a major step forward for gene selectionism because it ensures that the entities that are identified as replicators have a direct correspondence with some observable phenotypic character. The reasons for why these observable phenotypic characters, including complex adaptations, come about can then clearly be attributed to the differential selection of individual replicators! By requiring that replicators assert some sort of phenotypic power, Dawkins effectively preserves the explanatory power of the original replicator/interactor distinction.

So far so good, but it is the *next* step that Dawkins takes that is problematic, as Dawkins argues that *genes* qualify as these replicators that have the additional property of a consistent phenotypic effect on the world: "Unlike a nucleotide, a cistron is large enough to have a consistent phenotypic effect, relatively, though not completely, independently of where it lines on the chromosome (but not regardless of what other genes share its genome)." (*The Extended Phenotype* 92) To evaluate this claim, it is first necessary to understand exactly what Dawkins means by gene, or *cistron*. A cistron is a fragment of DNA which shows a certain behavior in a specific genetic test called a cis-trans complementation test. The details of this test are not important, but what Dawkins stresses throughout his argument is that the cistronic genes are fragments of DNA that cause *differences* between two phenotypes. This abstract Mendelian conception of the gene has been at the center of classical genetics long before the molecular revolution: genes are not necessarily assigned to any particular DNA sequence, but are simply

the units which segregate according to Mendel's laws of inheritance, describing *differences* in phenotype as they appear across generations. As an example, Dawkins considers the differences in coloration of moths. If a certain dark coloration pattern seems to be taking over a population of otherwise lightly colored moths, it is the DNA fragment that causes the *difference* between these two phenotypes which should be called a gene. So Dawkins conveniently defines genes by their consistent difference-making phenotypic effects! Fair enough, but if we closely examine what the DNA fragments that cause these phenotypic differences actually look like, Dawkins's view begins to run into trouble.

Dawkins claims that the difference between the light and dark moth phenotypes can be due to a difference at a single locus, even though there are many genes that contribute to both of the phenotypes. Certainly, there are times in which this is the case. For instance, the difference between the wrinkled and smooth phenotypes in Mendel's famous peas is due to differences in a locus that contains a sequence of DNA that codes for a single protein product, the starch branching enzyme. Loss of a certain isoform of this enzyme results in dehydrated, wrinkled peas (Smith 1988). Competition between different versions of replicators located at the starch branching enzyme locus, with each of the replicators exhibiting their difference-making phenotypic power, accurately explains the interplay between these two phenotypes. But the vast majority of trait differences are definitively *not* due to simple differences of alleles at a single locus. Differences in height, for instance, cannot be attributed to differences in a single protein-coding DNA fragment or any other individual locus. Rather, the genetic elements responsible for these trait differences are scattered throughout the genome. According to Dawkins's view, the scattered collection of genetic elements responsible for height differences should be called a single *gene*, and would qualify as a single replicator. But clearly, this cannot be the case, since

fragments of DNA scattered throughout the genome do not form a single *lineage*, as they will be shuffled around each generation during crossing over. This same objection to Dawkins's view has been realized by Sterelny and Griffiths (1999), who show how differences in a phenotype as simple as human eye color must be attributed to many different loci which, together, do not form a single active replicator lineage. Dawkins seemed to be placing an empirical bet that most trait differences could be attributed to cohesive fragments of DNA, a bet which we now know he cannot win.

The properties of Dawkins's active replicator and his Mendelian conception of a gene clearly do not match up, since Mendelian genes often do not form unified, long-lived lineages. We have already seen that properties Dawkins ascribes to active replicators, including consistent phenotypic power, are useful properties for the replicator concept because they contribute to the explanatory power of the replicator/interactor distinction. The rest of this paper, then, will seek to identify the types DNA sequences which *do* qualify as Dawkins's active replicators. Thus, I am adopting the replicator/interactor distinction and Dawkins's active replicator concept, but rejecting the second principle of gene selectionism that claims that genes are the active replicators. But before we completely bury gene selectionism in favor of *replicator* selectionism, we should check to see if *molecular* genes can be considered replicators.

Molecular genes are fragments of DNA that code for a single mRNA transcript. Although there was hope that the Mendelian version of the gene as a difference maker would eventually line up with the molecular gene, the same difficulties of mapping phenotypic differences to particular genetic elements resulted in the reduction not working out (Griffiths and Stotz 2013). As all molecular genes are limited to a single short stretch of DNA, they do form long-lived lineages, unlike Mendelian genes. However, while Mendelian genes had consistent phenotypic

effects, it has recently become clear that the phenotypic effects of molecular genes on an organism, even at a molecular level, are far from consistent. While it was once thought that a single molecular gene codes for a single protein with one particular function, we now know that the majority of molecular genes produce a wide range of protein products. This one-to-many relationship between molecular genes and proteins is made possible by a range of posttranscriptional processing mechanisms a cell has at its disposal, including alternative splicing and RNA editing, which will be described in more detail later. By producing an array of protein products that perform different functional roles leading to different phenotypic characteristics, it is clear that molecular genes don't have consistent phenotypic effects, and thus don't qualify as active replicators.

A central problem for the proponents of the replicator/interactor distinction has been the inability to convincingly identify the specific DNA fragments across species that qualify as active replicators. While the arguments in favor of the replicator/interactor distinction are strong in principle, it has been difficult to see the utility of the distinction in practice without robust identification of the active replicators. In the following sections, then, I will survey the types of DNA fragments that *do* qualify as active replicators according to Dawkins's criteria: parts of entire chromosomes of asexually reproducing organisms, plasmids, transposons, protein domain-coding regions, and regulatory elements. With each type of replicator, I will attempt to show not only how replicator selectionism helps explain the features of the organisms the replicators reside within, but also how this view illuminates interesting differences in the evolutionary dynamics between different organisms. Let's begin our journey by examining the active replicators present in asexually reproducing organisms.

## 2. Active replicators in asexually reproducing organisms include parts of bacterial chromosomes, plasmids, and transposons.

### 2.1 Bacterial Chromosomes

Recall that longevity, or the ability to form a potentially immortal lineage of copies, is an essential property of an active germ-line replicator. If no internal shuffling of DNA fragments due to recombination or genetic crossover along a stretch of DNA takes place, entire DNA molecules could be considered active germ-line replicators. In asexually reproducing organisms, including bacteria, this is precisely what happens. Prior to each cell division, the entire genome of the bacterium, housed in a single DNA molecule called the *bacterial chromosome*, is directly copied without any recombination-induced internal shuffling. Dawkins supports the idea that the entire bacterial chromosome of bacterial cells should thus be considered a single active replicator (1978); in addition to forming a lineage, the bacterial genome produces a consistent phenotypic effect of a nearly identical organism every generation. Upon closer examination of the composition of a bacterial chromosome, however, I will argue that we must amend this position slightly. As is the case with most genomes, bacterial chromosomes are littered with DNA fragments called transposable elements, or transposons. Transposons are small fragments of DNA that have the remarkable ability to be spliced out or copied from the DNA at one location, and reinsert themselves at another location on the same DNA molecule, or on a different DNA molecule altogether. In bacteria, transposons can jump in and out of the bacterial chromosome, or between the bacterial chromosome and bacterial *plasmids*. Plasmids are small circular pieces of DNA which are physically separate from the bacterial chromosome, carrying a few molecular genes which often confer some advantage to the bacterial cell, such as antibiotic resistance. We will examine the evolutionary dynamics of plasmids in greater detail shortly, as they should also

be considered active replicators, but for now it is important to note that some plasmids can integrate into the bacterial chromosome under the right conditions, just like transposons. Now, if we view the entire bacterial chromosome is an active replicator, we are including the transposable elements and plasmids that happen to be present in the chromosome at the time. But some transposons and plasmids inevitably leave the chromosome while others are inserted every generation. They are not consistently copied as a single unit, and thus do not form a lineage; a collection of transposons within a bacterial chromosome cannot be considered part of a single active replicator. Remember that longevity and lineage-formation is an essential property of an active replicator because if the DNA sequences within a replicator do not stick together as a unit, cumulative selection cannot act on those sequences as a unit. In addition, many bacterial transposons, just like plasmids, allow their host bacterium to become resistant to certain antibiotics. With these two genetic elements coming and going from the bacterial chromosomes, the phenotypic effect of a single chromosome is *not* consistent. In some generations, part of the chromosome's effect is resistance to penicillin, and in other generations, that resistance is abruptly lost. In light of these problems resulting from considering the entire bacterial chromosome an active replicator, I propose to consider the bacterial genome *minus* transposable elements and plasmids as an active replicator. This way, only the parts of the bacterial chromosome which satisfy the requirements of being consistently copied and forming a lineage are considered part of the active replicator. Now, the bacterial chromosome may be *broken up* by the insertion of transposons into various loci every generation. But this does not result in the various parts of the chromosome being *separated* from one another; the chromosome is still copied as a single DNA molecule, with all the sequences innervated by transposons and plasmids selected as a single unit.

At first glance, the evolutionary dynamics of differential selection of bacterial chromosomes might seem relatively straightforward: the versions of chromosomes that build more successful bacteria will be selected for and increase their prevalence in the population. The same is true for plasmids and transposons, which are replicators in their own right, as they confer some sort of consistent advantage to the bacterial cell in which they reside while forming a lineage of copies. As such, we can explain how the complex adaptations of bacterial cells arise by appealing to the accumulation of slight modifications of either the bacterial chromosome or a certain plasmid, depending on whether the genetic component of the adaptation is based in the chromosome or a plasmid. What complicates things substantially is that in this view of bacterial evolution, the relationship between active replicator and phenotypic characteristic or even molecular characteristic is not one-to-one. The differential selection of *the same replicator* will explain the appearance of multiple bacterial features if the coding sequences that produce the proteins involved in both processes are located on the bacterial chromosome. But remember that an active replicator need not have a one-to-one relationship with its phenotypic or molecular effect, but rather a *consistent* effect. Although a bacterial chromosome affects a whole range of different physical bacterial processes, its effect is nevertheless relatively consistent, no matter which bacterial cell or which generation the same chromosome finds itself in. The chromosome produces a fundamental interactor, the organism, which responds and reacts to the environment in certain ways.

Let's take a brief look at a bacterial feature to see how an evolutionary explanation based on the differential selection of active replicators might go. One very interesting adaptation that most bacteria possess is *quorum sensing* (Ryan and Washburn 2014). This is a phenomenon by which bacteria are able to sense and respond to the local population density by synthesizing and

secreting small molecules called *autoinducers*, recognizing the concentration of these autoinducers by binding between the autoinducers and receptors, and responding to this signal by changing gene expression patterns (Figure 1). When there is a high concentration of bacteria, the concentration of the secreted autoinducers rises as well, since there are more bacteria around secreting this molecule in a smaller area. Bacteria can sense this high concentration of autoinducers, as more of their autoinducer receptors are in the bound state. Then, depending on the bacterial species, the recognition of the autoinducer leads to coordinated group behaviors between the bacteria in the area, including biofilm formation and bioluminescence. In fact, many species of marine organisms, including the Hawaiian Bobtail Squid, rely on the symbiotic relationship with bioluminescent bacteria, illuminated by quorum sensing, to avoid predators and sneak up on unsuspecting prey.

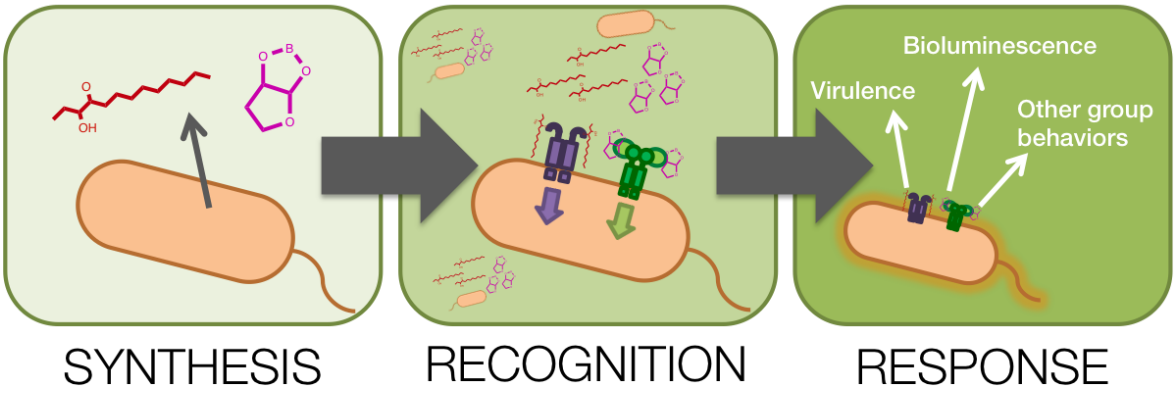


**Figure 1.** Quorum sensing in bacteria consists of synthesis and secretion of autoinducers, recognition of autoinducer concentration, and a response based on altered gene expression patterns (from Ryan and Washburn 2014).

Surprisingly, the molecular details of quorum sensing that lead to bioluminescence in the species of bacteria that live endosymbiotically with the bobtail squid, *V. Fischeri*, are not too complicated and worth examining here in some detail so we can explain how the phenomenon might have evolved (Figure 2). First, the enzyme *LuxI* synthesizes an autoinducer, in this case a

molecule called *acetylated homoserine lactone* which can diffuse freely across the cell membranes of this species of bacteria. *V. Fisheri* also contains a receptor protein that recognizes and binds the autoinducer (*LuxR*). Upon this binding interaction, the autoinducer/receptor complex binds to a regulatory region in the chromosome ahead of the set of molecular luciferase genes whose gene products cause bioluminescence. Now, there is no reason for an individual *V. Fischeri* bacterium to waste precious energy producing luciferase gene products if there are no other bacteria around, because a high density of bacterial cells are required to produce enough light to confer an adaptive advantage to the squid host (Ruby 1996). Quorum sensing ensures that the bacteria only glow in the presence of other bacteria, when illumination actually confers a selective advantage.

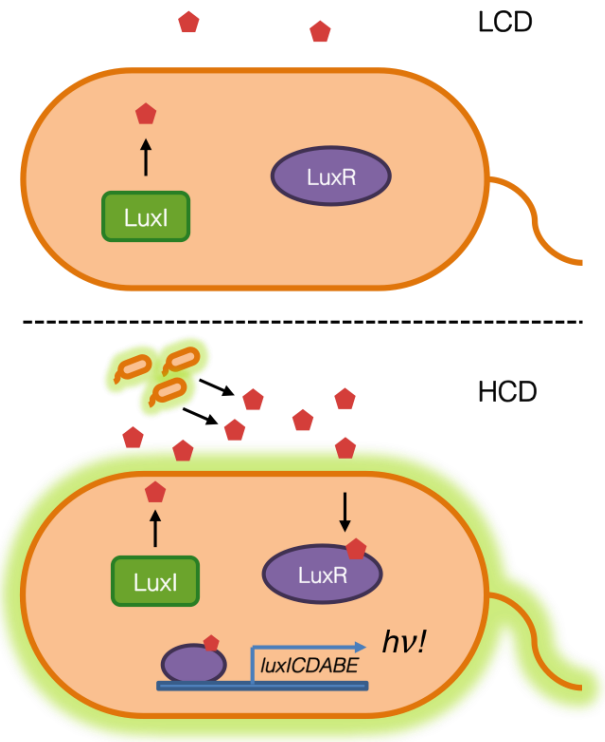


**Figure 2.** Molecular details underlying quorum sensing in *V. Fischeri.* LCD= low cell density HDC= high cell density. See text for details (From Ryan and Washburn 2014).

How might we use the tools of replicator selectionism to explain how quorum sensing evolved in *V. Fischeri*? First, we must identify the active replicator responsible for the effects we see in the interactor. In this case, the molecular genes that code for the two proteins that do the work in the system, *LuxI* and *LuxR*, are both located in bacterial chromosome (Fuqua *et al* 1994), so the phenotypic effects caused by *LuxI* and *LuxR* are part of the phenotypic power of the bacterial chromosome. Now, different bacterial cells in a population will have slightly different versions of the *LuxI* and *LuxR* genes; every time a bacterial cell reproduces and copies these genes along with the rest of the chromosome, there is a small chance that a mutation is introduced into these genes that affects the function of the gene products. Let's imagine that during one fateful cell division in the early evolution of quorum sensing, a small modification in the coding region of the *LuxI* gene was introduced that allowed the *LuxI* gene product to synthesize more autoinducer. After many subsequent cell divisions, two populations of cells would emerge: one population with the old *LuxI* gene, and one with the new modified version. The bacterial populations with the modified replicators would be able to offer the host squid better protection from predators, as groups of bacteria might glow at slightly lower concentrations. There would be differential selection of these bacterial interactors, then, since the squid containing bacteria with old versions of the *LuxI* gene might be more likely to be eaten by predators. Over many generations, the successful active replicators were the ones that accumulated many small modifications in the *LuxI* gene that resulted in the preferential survival of the interactors that conferred better bioluminescence to the bobtail squid while not wasting energy producing luciferase when other bacteria were not around. But we must remember that it is not the *LuxI* gene that is an individual replicator, but rather the *entire* chromosome. Thus, the cumulative selection of the *LuxI* gene must actually be *paired with* the cumulative selection of

the *LuxR* gene. Beneficial mutations in *both* genes must accumulate *at the same time* in order for quorum sensing to evolve; if mutations in *LuxI* causes the bacteria to begin secreting autoinducer without the coevolution of the receptor protein, there will be no positive selection pressure for these *LuxI* genes. Of course, this paired accumulation of beneficial mutations only happens in a vanishingly small number of bacterial chromosomes. But remember that bacteria divide at a furious rate, allowing this cumulative selection to happen much more quickly than in larger, sexually reproducing organisms with longer life cycles.

There are drastic differences in evolutionary dynamics between sexually reproducing organisms and asexually reproducing organisms due to the size of the active replicators; since bacterial replicators encompass many molecular genes, coevolution of paired beneficial mutations in asexually reproducing organisms is required. To see this difference clearly, it is helpful to expand an analogy first developed by Dawkins in *The Selfish Gene* (1976). Consider the active replicators in sexually reproducing organism members of rowing teams, with winning boats with the best rowers able to "reproduce" their rowers and continue racing (Dawkins thinks the active replicators are genes, but as we shall see they are in fact protein-domain coding fragments; however, the actual nature of the replicator does not matter here). Now, in the racing dynamics corresponding to sexual reproduction, the teams of rowers in the winning boats are shuffled around every generation, corresponding to the genetic shuffling that occurs during both crossing over *and* the independent segregation of chromosomes. Thus, successful rowers must be able to perform well with a variety of other teammates, and the really poor rowers are quickly weeded out as they drag every boat down they are part of. In the races with boats representing asexual reproduction, however, no such shuffling occurs after every race. Instead of each rower being individually selected over the long run, since they are part of many different teams, *entire*

*teams of rowers* are selected. So there might be a fantastic coxswain in a certain boat with a terrible stern rower, but since this coxswain never ends up with any other teammates, the coxswain never ends up in winning boats and thus does not effectively increase her prevalence in a population. In this team of rowers that stick together race after race, it can be said that there is a combination of positive selection for the coxswain but negative selection against the stern rower, resulting in some average selection pressure.

By acknowledging that much smaller fragments of DNA are replicators in sexually reproducing organisms than in asexually reproducing organisms, the advantage of sexual reproduction over asexual reproduction in producing complex adaptations through cumulative selection becomes readily apparent. Since the entire team of molecular genes acts as a single replicator in a bacterial chromosome, small advantageous mutations in one molecular gene are often dragged down by deleterious mutations elsewhere in the chromosome. Thus, the cumulative selection for advantageous versions of certain molecular genes is much more difficult than in sexually reproducing genomes, as there is less of a guarantee that a small advantageous modification will lead to an increase in the fitness of an interactor when the advantageous modification is stuck on a huge chromosome. In sexually reproducing organisms, on the other hand, a small advantageous mutation in a replicator that is unlucky to be located on a genome with relatively poor overall fitness one generation will get a chance to be paired with a variety of other teammates in subsequent generations due to meiotic crossover, provided that the original organism doesn't die before reproduction. Interestingly, most of the rapid evolution seen in bacteria is actually based on modifications in a much smaller replicator: plasmids (minus their transposable elements of course, as with the bacterial chromosome) (Svara and Rankin 2011).

## 2.2. Plasmids

Plasmids do not suffer the same restrictions that bacterial chromosomes face for developing advantageous phenotypic characteristics for their interactors through cumulative natural selection. For one, plasmids are much smaller than bacterial chromosomes, so an advantageous variant of a certain molecular gene is not as likely to be paired with another disadvantageous mutation in a second molecular gene on the same plasmid. But more importantly, plasmids can be paired with a whole range of different teammates in the form of bacterial chromosomes and other plasmids because of the process of bacterial conjugation. In bacterial conjugation, a plasmid is replicated while two bacteria are physically connected, with one plasmid copy remaining in the original bacterium and the other copied transferred to a new host bacterium. Since a plasmid can exert phenotypic power on a number of different interactors, there can be rapid cumulative natural selection on bacterial plasmids, which helps explain why the rapidly evolving antibiotic resistance phenotypes that both appear quickly and spread quickly throughout bacterial populations are commonly found on plasmids, not on bacterial chromosomes.

## 2.3 Transposons are active replicators in both asexually and sexually reproducing organisms

The other type of active replicator that often causes antibiotic resistance in bacteria is the transposon. Again, just like plasmids, transposons are relatively short sequences of DNA and have the ability to jump around and play with many different teammates, explaining why many bacterial characteristics based on transposons are quick to evolve and spread throughout a population. Transposable elements are ubiquitous in the genomes of sexually reproducing organisms as well, but they rarely code for any proteins that end up causing a phenotypic effect

as Tn transposons do in bacteria. Instead, many *eukaryotic transposons* contain genes coding for a *reverse transcriptase,* an enzyme that converts RNA into DNA. These types of transposons, called retrotransposons, have the remarkable consistent molecular effects of both recruiting DNA polymerase to transcribe the transposon sequence into RNA, *and* causing the resulting RNA transcript to be converted back into DNA by virtue of the reverse transcriptase. The transposon has effectively copied itself without the use of any DNA replication machinery, with the copies of the transposons free to integrate to other sites of the host genome.

The horizontal replication realized by transposons results in fascinating evolutionary dynamics which are very different from the other replicators we examine here. By only producing a reverse transcriptase that specifically reverse transcribes its own RNA, retrotransposons rarely influence the fitness of the sexually reproducing organisms they reside in (although sometimes, transposons that integrate in the middle of an important molecular gene might cause deleterious effects) (Cox *et al* 2012). Differential selection of organisms does not explain the differential selection of transposons, and organisms do not qualify as the interactors of these active replicators. Instead, it is both the reverse transcriptase protein product of retrotransposons *and* the retrotransposon sequence itself that qualify as interactors!

To see why this is, let's first imagine that there was a slight modification to the coding sequence for the reverse transcriptase within a transposon located at a certain locus. The modification allows the reverse transcriptase to reverse transcribe the target transposon RNA at a higher rate, resulting in more DNA copies of the transposon than the normal, unmodified reverse transcriptase produces. With a higher rate of replication in the modified transposon than in the original transposon, the modified transposon will eventually increase its representation in host genomes by integrating in more places than the original transposon sequence. The reverse

transcriptase, then, was the entity whose interaction with the cellular environment caused the differential selection of the two versions of the transposon, and qualifies as an interactor. Alternatively, there could be a slight modification in the inverted repeats of a transposon. Inverted repeats are sequences that help reverse transcribed DNA copies of the transposon integrate into new sites in the host genome. A modification in these inverted repeats of a transposon at a certain locus might allow for more efficient integration of the copies of this modified transposon than for the copies of the original transposon. As a result, the copies of the modified transposon will increase its prevalence in the genomes because copies of the replicator itself are more successful at surviving by integrating into the host genome[2]! Again, we see how replicator selectionism highlights interesting and important differences in the evolutionary dynamics between different genetic elements. Identifying transposons as active replicators in both asexual and sexual genomes allows us to see that their cumulative natural selection is completely independent of the selection that takes place of other active replicators in their host organisms, and that interaction between the actual transposon sequence and the cellular environment causes the differential selection and explains the evolution of these sequences.

Interestingly, the ubiquitous retrotransposons present in genomes of sexually reproducing organisms also play important roles in explaining the evolution of phenotypic characteristics in these organisms. In humans, a whopping 1.5 million short retrotransposons known as Long-interspersed nuclear elements (LINEs) make up approximately 15% of the genome (Lodish *et al* 2000). The vast regions of the genome full of LINEs had for many years been considered

[2] Although transposons (along with regulatory elements) might be unique among replicators today in being both replicators and interactors, it is likely that life may have gotten off the ground by replication of entities which were both replicators and interactors. In the RNA world hypothesis (Higgs and Lehman 2015), fragments of free-floating catalytic RNA promote their own replication before natural selection favored the creation of separate interactors.

nonfunctional “junk” DNA, but recently, it is becoming clear that most of these segments actually play some functional role. The Encyclopedia of DNA Elements (ENCODE) project, which aimed to identify all the functional elements of the human genome, recently concluded that 80% of the genome is associated with at least one biochemical function (Bernstein *et al* 2012). So the LINEs and many other retrotransposons probably first integrate into our DNA as individual active replicators, solely influencing the probability that they are copied to other regions of the genome. Eventually, however, many of these retrotransposons are usurped by the genomic machinery, and gain important biochemical functions. For instance, retrotransposons often become part of enhancer or insulator sequences that affect the transcription rate of molecular genes located many kilobases away or even on different chromosomes. As soon as this integration happens, the differential survival of the organism will result in the differential survival of slightly different versions of the retrotransposons that differentially affects the transcription rate of a certain molecular gene.

The retrotransposon is now part of an active replicator called a *regulatory element* (see part 5), which has a consistent phenotypic effect of increasing or decreasing the expression level of a set of molecular genes. Cumulative selection of the retrotransposon can now result in changing expression levels for certain molecular genes, which in turn leads to changes that eventually result in complex adaptations. So initially, transposons do not play an important role in explaining any cumulative selection that leads to observable phenotypes in sexually reproducing organisms, because the only consistent effect they have is to recruit cellular machinery to copy themselves to other parts of the genome. But, when they gain a functional role in the context of gene expression, competition between different versions of the transposon located at a specific locus can lead to cumulative selection of a regulatory element.

Identification of parts of bacterial chromosomes, plasmids, transposons, and regulatory elements as active replicators shows how replicator selectionism is a useful tool for studying evolutionary dynamics. Notice that by identifying active replicators, we also reach satisfying answers to the question of *who benefits* from the characteristics we looked at: plasmids are the ultimate beneficiaries of antibiotic resistance, chromosomes benefit from quorum sensing, and transposon DNA sequences benefit from the action of transposon reverse transcriptase. Next, we will see how protein-domain coding fragments of DNA and regulatory elements help explain complex adaptations and evolutionary dynamics in sexually reproducing organisms.

## 3. Active replicators in sexually reproducing organisms must have consistent *molecular* effects

Sexual reproduction is a game-changer. Through crossing over and differential pairing of active replicators in every generation of diploid sexually reproducing organisms, sexually reproducing organisms can try out many combinations of active replicators, greatly facilitating the appearance of complex adaptation. To see how differential selection plays out in sexually reproducing organisms to produce this complex adaptation, we must begin as before by identifying the active replicators. Soon, we will see how individual fragments of DNA coding for protein domains *and* individual fragments called regulatory elements are the active replicators in sexually reproducing organisms. In order to identify these replicators in sexually reproducing organisms, we must look for those fragments that have consistent phenotypic effects on the *molecular* level, instead of consistent phenotypic effects on the level of the organism, as we saw for bacteria. This move requires justification. In this section, then, I will explain why consistent molecular effects in sexually reproducing organisms are equivalent to consistent phenotypic

effects on the level of the organism, and show why it is important to require that active replicators still have consistent molecular effects.

In sexually reproducing organisms, the entire genome (minus transposable elements) does not qualify as an active replicator as it does in asexually reproducing organisms because the genome is not located on a single DNA molecule. Instead, the genome is divided among many chromosomes which segregate independently during meiosis. As a result, chromosomes aren't necessarily inherited together and they cannot form a lineage and by selected *as a unit*, which is an essential feature of active replicators in Dawkins's framework. Individual chromosomes cannot form lineages either because of the phenomenon of crossing over, as homologous chromosomes exchange fragments of DNA and break up chromosomes each generation. How small must active replicators be in order to safely avoid the fragmenting effects of crossing over and form a lineage? In turns out that no fragment we pick as a candidate replicator greater than 1 nucleotide long is completely safe from crossing over. Although crossing over usually occurs at certain recombination hotspots located between coding regions of the chromosomes, the boundaries of recombination events *can* be located between any two nucleotides. Thus, a lineage formed by any fragment of DNA greater than 1 nucleotide in length will eventually be split apart by crossing over, whether that fragment is 2 nucleotides long or an entire chromosome.

We seem to have arrived again, by a different route, at the absurd reduction that a single nucleotide qualifies as an active replicator. As we saw before, this is a conclusion that we want to avoid at all costs, since there is no meaningful competition between individual nucleotides. The solution Dawkins proposes to this problem is to relax the stringency on the requirement that the lineage formed by replicators must be *immortal*. Dawkins argues that there is no reason that the longevity of an active replicator must be indefinite. Instead, in order for an active replicator

to be selected as a unit, the longevity must simply be long enough for selection pressures to cause some differential selection of different versions of the replicator. So for instance, if the replicator is broken up every 5 generations on average, even a very strong selection pressures will not allow differential selection between different versions of this replicator. A small fragment of DNA of a few hundred nucleotides, however, will likely be copied as a unit for millions of generations before crossing over splits it up, allowing even weak selection pressure to act on the fragment as a unit. As Dawkins puts it, there is no "hard and fast" rule (Extended Phenotype 89) for how short a portion of the chromosome must be before it we can usefully consider it a candidate replicator. We don't *need* a precise rule, because the criterion of consistent phenotypic effect will help us identify exactly which kind of small DNA fragment of the many fragments that are candidates *actually* qualify as active replicators.

When identifying active replicators in bacteria, I argued that the entire bacterial chromosome without its transposable elements qualified as an active replicator in part because it has the relatively consistent phenotypic effect of producing more or less the same interactor every generation. Of course, the environment may then act upon the individual bacterial cells, resulting in bacterial cells with the same genome that have very different phenotypes; however, the initial input of the active replicator into the interactor is consistent for a bacterial cell, so we can say that the bacterial chromosome has a consistent phenotypic effect. There is a simple and direct correspondence between the replicator and the phenotypic characteristics of the organism, namely, that almost every phenotypic character can be attributed to differential selection of the bacterial chromosome. Now, when we move to genomes that are affected by crossing over, things get *much* more complicated. The active replicators in sexually reproducing organisms must be significantly smaller than an entire chromosome in order to form a lineage. As a result,

these replicators contribute only a small part of the overall observable phenotype of the organism. Looking at the level of the organism, there is often no longer a direct correspondence between the candidate replicators and any observable phenotypic effect on the level of the organism. Any relatively small fragment of DNA, whether it is a group of molecular genes, a single molecular gene, or even a protein-domain coding fragment works together with other candidate replicators to produce the characteristics of the final organism, along with significant input from the developmental environment.

Recall that one reason why the additional feature of phenotypic power is a useful feature to require of replicators is that a direct correspondence between replicators and phenotypic characteristics follows. The appearance of phenotypic characteristics can then be explained by the differential selection of the corresponding replicators. In the case of sexually reproducing organisms, it seems that no direct correspondence exists between phenotypes of interactors and the relatively small active replicators. The direct correspondence does exist, however, if we consider *smaller parts* of whole organisms as individual interactors.

An interactor is any entity which is built by active replicators and causes the differential selection of the replicators while acting as a cohesive whole. In unicellular asexually reproducing organisms, it is clear that the entire organism is the only interacting entity, since the differential selection of the organism causes the differential selection of the single replicator that is responsible for most of the phenotypic characteristics, the bacterial chromosome. In sexually reproducing organisms, active replicators are significantly smaller, and can produce interactors that interact *with the environment inside the organism* to cause the differential selection of the active replicators. In these organisms with collections of small active replicators, then, there is a *hierarchy of interactors* that work together strategically to build whole organisms. Proteins

occupy the lowest level of this hierarchy (which I call the *fundamental interactors*) with differential selection of successful proteins in the cellular environment influencing the active replicators that code for parts of proteins. Proteins cooperate to produce larger interacting entities, including organelles and cells. I will come back to the hierarchy of interactors and exactly how differential selection of low level interactors plays out when I explain how active replicators can explain complex adaptations in sexually reproducing organisms. For now, it is enough to understand that these low-level interactors exist. Let us get back to identifying those active replicators in the first place.

The way we can see direct phenotypic effects of active replicators in sexually reproducing organisms is by looking at the phenotypic effects of these replicators on interactors that lie *low* on the hierarchy. For instance, if molecular genes are our candidate replicators, we can test to see if they have consistent phenotypic effects by determining whether they produce consistent effects on certain kinds of proteins. In this case, all the phenotypic characteristics of a certain protein do correspond directly to a single stretch of DNA, namely the molecular gene from which the mRNA was transcribed and then translated. But as noted previously, molecular genes do not consistently produce protein interactors with the same phenotypic characteristics. Changes to the mRNA transcribed from a molecular gene, called posttranscriptional processing, results in molecular genes producing a whole array of different protein products depending on the cellular context. But even though the phenotypic effect of molecular genes on interactors is not consistent, we still see the direct correspondence between interactor and replicator that makes the replicator concept so useful. Why require a consistent phenotypic effect, and not just direct correspondence in this case?

It turns out that there is a *second* major advantage to requiring active replicators to exhibit a consistent phenotypic effect, an advantage that is only observed in eukaryotic organisms that have mechanisms of modifying mRNA before it is translated to proteins so that a single fragment of DNA can produce a variety of interactors. By requiring a consistent phenotypic effect, we can rule out cases in which there is a *one-to-many* relationship between active replicators and functions of their interactors, as is the case with molecular genes and their array of protein products with different functions depending on the set of posttranscritptional processing enzymes present at the time. This one-to-many relationship between DNA sequences of replicators and the functions of their corresponding interactors is very problematic because the same DNA fragment experiences many different types of selection pressures at once if its gene products have many different functions. It becomes quite difficult to see how cumulative selection could culminate in the function of a particular protein product if each small modification in the replicator is simultaneously affecting the function of many other related proteins.

It will be useful to prove this important point, that active replicators must not have one-to-many relationships with functions, with an example. One famous example of alternative splicing, a mechanism of producing many proteins from a single mRNA transcript that will be explained in greater detail soon, involves a molecular gene that codes for two peptides simultaneously: calcitonin, and calcitonin-related-gene-related peptide (CGRP), each with distinct functions. Calcitonin is expressed in the thyroid and helps regulate calcium levels, while CGRP is expressed in neurons and is a potent vasodilator. Now, when we look to explain how these functions evolved, we look to the differential selection of versions of replicators responsible for these functions, which *in each case* is the calcitonin gene. So we cannot say that

small modifications in the active replicator, the calcitonin gene, were simply differentially selected for over many generations through the success of its interactor, calcitonin, because the evolution of the calcitonin gene is *also* affected by the differential selection of the CGRP interactor. Small modifications beneficial to calcitonin interactor might very well be deleterious to the CGRP interactor, and there is no way to see *which* modifications might be advantageous to *both* interactors *unless* we look at where exactly the modifications take place in the DNA sequence (i.e., in which domain-coding fragment). We must look at how units *smaller* than the molecular gene have consistent phenotypic effects in both the CGRP interactor and the calcitonin interactor to adequately explain the differential selection that takes place within the calcitonin gene.

Dawkins does not seem to think it is a problem that a single replicator has multiple phenotypic effects. In fact, his famous *greenbeard gene* is *pleiotropic*, meaning that it affects a number of different traits directly; greenbeard genes both cause organisms to grow green beards *and* increase the propensity of an organism to help organisms that have green beards. Of course, Dawkins is talking about a Mendelian greenbeard gene, which includes all the genetic elements responsible for the differences in these traits, so Dawkins's greenbeard genes could include a number of molecular genes scattered throughout the genome. But let's assume that the greenbeard gene *was* in fact a molecular gene, producing a number of polypeptides through posttranscriptional processes. We could do some hand-waving and say that there was cumulative selection of modifications in the greenbeard gene that benefitted the whole group of proteins, each with distinct functions. But as in the case of the calcitonin gene, it is not clear what selection pressures are present on a small modification in the greenbeard gene. We can do much

better than this by looking at the components of molecular genes that have consistent molecular effects.

## 4. Domain-coding fragments are active replicators in the coding regions of sexually reproducing organisms, and differential selection of these domain-coding fragments through a hierarchy of interactors explains the evolution of features of sexually reproducing organisms.

Now that I have shown why active replicators in sexually reproducing organisms need to be relatively small (due to crossing over) and why they must have consistent *molecular* effects (due to the one-to-one relationship with the function of an interactor that follows), I am finally in the position to argue that individual DNA fragments that code for protein domains and regulatory elements are active replicators in sexually reproducing organisms. We will begin be looking at domain-coding fragments. I will first explain what exactly a protein domain is, before showing how differential selection between different versions of protein domains can produce complex adaptation through a hierarchy of interactors.

### 4.1. Protein domains

As is the case with the gene concept, a protein domain does not have a single consensus definition in the scientific literature, so the precise definition of the protein domain depends on where the term is used. The concept of a protein domain was first introduced in 1973 as a part of a protein which could fold independently of the rest of the chain (Wetlaufer 1973). Since then, the term has come to denote a structurally, functionally, and evolutionarily *independent* part of a protein (Guo *et al* 2003). Structurally, a protein domain remains intact in its natively folded state if it is cleaved from the protein, and should appear structurally similar if the same domain is

present in multiple proteins. The specific molecular function of a protein domain should also remain constant over all the proteins which contain the domain. Finally, the nucleotide sequences that code for domains are often highly conserved and considered evolutionary 'modules' of proteins. For our purposes, the most important feature of a protein domain is that its function in the context of the protein is consistent. That is, it plays the same role in catalyzing a reaction or interaction between molecules no matter where the domain appears in the proteome. During the past 50 years, researchers have conveniently identified and characterized tens of thousands of functionally independent units of proteins, without realizing that they were also describing fundamental units of selection in sexual genomes!

Now, just as discrete molecular genes code for proteins, discrete fragments of DNA which I refer to as *domain-coding fragments* code for individual protein domains. The phenotypic effect of these domain-coding fragments on proteins, the fundamental low-level interactors, is to provide the molecular function of the protein domain that the fragments code for. So there is a direct correspondence between domain-coding fragments and the phenotypic effect of protein domains. But is the effect that the domain-coding fragment bestows on the replicator truly *consistent?* It turns out that contrary to the relationship between molecular genes and the many functions of the protein products, the relationship between domain-coding fragments and the phenotypic effects they bestow on their interactors, the protein domains, *is* one-to-one. This is because posttranscriptional editing processes that act on the mRNA before translation occurs do not affect the function of protein domains. To see why this is the case, it will be necessary to take a closer look at the major posttranscriptional editing processes present in the cell that can alter the final protein sequences, especially alternative splicing and RNA

editing, and see why they result in many functions for molecular genes but only a single function for domain-coding fragments.

To facilitate this investigation into posttranscriptional editing processes and consistent phenotypic effects of domain-coding fragments, it will be useful to consider a specific example of a typical protein domain: the Scr homology-2 domain, or SH2. Although technically a protein domain, SH2 is commonly (and confusingly) referred to as an *adaptor protein* because it functions to mediate interactions between two or more proteins. SH2 appears in hundreds of different proteins involved in cell signaling processes that proceed through a certain, specific type of signal receptor protein that spans the cell membrane, called a Receptor Tyrosine Kinase (RTK) (Figure 3.1). When a signaling molecule binds the extracellular domain of the RKT, the RTK becomes activated and can relay the binding signal to the inside of the cell. The SH2 domain has a high affinity for phosphorylated tyrosine residues that are part of an activated RTK, and *functions* to bind and keep whichever protein it is part of (a *secondary messenger protein*) in close proximity to the RTK (Figure 3.2). One second messenger that includes an SH2 domain is Growth-factor receptor bound protein 2 (Grb2). As its name suggests, the SH2 domain helps activate Grb2 by facilitating binding with the transmembrane growth factor receptor. Grb2 also has a different protein domain called *SH3*, which functions to bind a proline-rich motif in the protein *SOS*, a downstream target of the signaling pathway. By using two adaptor domains, Grb2 is able to carry a signal between two specific proteins (Figure 3.3). Now, there are hundreds of *different* secondary messenger proteins in the cell that contain the *same* SH2 domain. In each of these proteins, the function of SH2 is the consistent: to facilitate binding between the RTK and the secondary messenger. This way, a single extracellular signal can reach a number of specific protein targets within the cell.

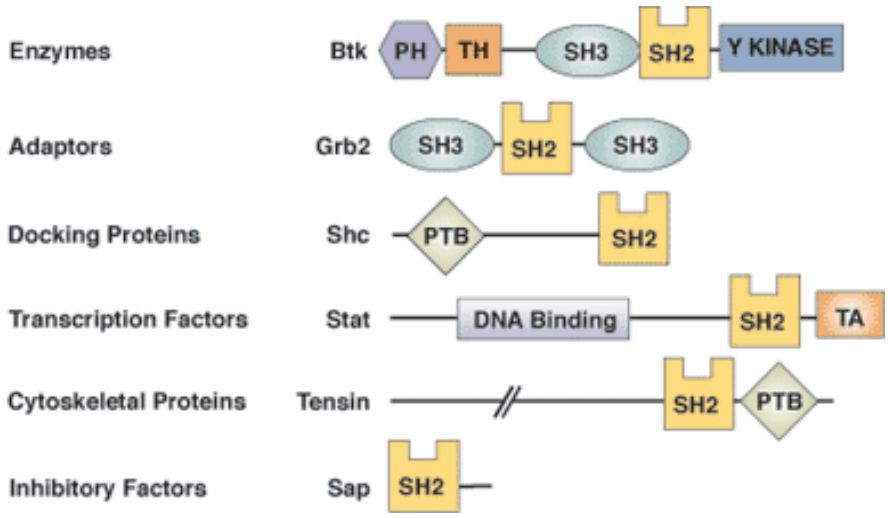


**Figure 3.1.** The SH2 domain is present in a many of different proteins with distinct functional roles in the context of the cellular environment. However, the molecular effect of the SH2 domain is consistent in each protein: to bind phosphotyrosine residues (From "SH2 Protein Domain: Domain binding and function." *Cell Signaling Technology*. n.d. Web, 4/20/2015).

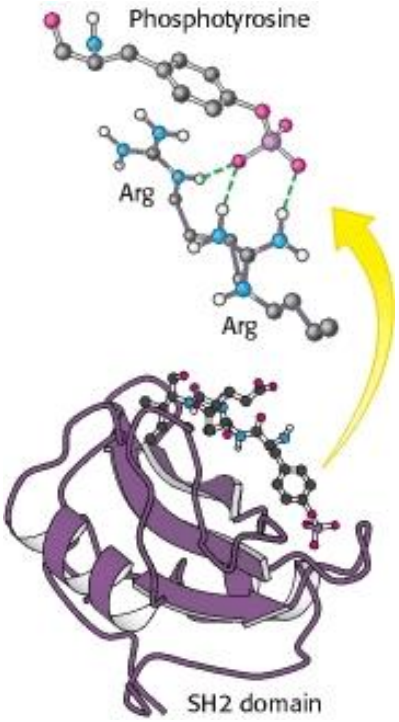


**Figure 3.2.** (left) A cartoon depicting the binding interaction that takes place between an SH2 domain and a phosphotyrosine residue at the molecular level. Notice that the amino acids of the SH2 domain (labeled Arg for Arginine) form a pocket that perfectly 'captures' and binds the phosphate group of the phosphotyrosine (in magenta) (From Stryer *et al* 2002).

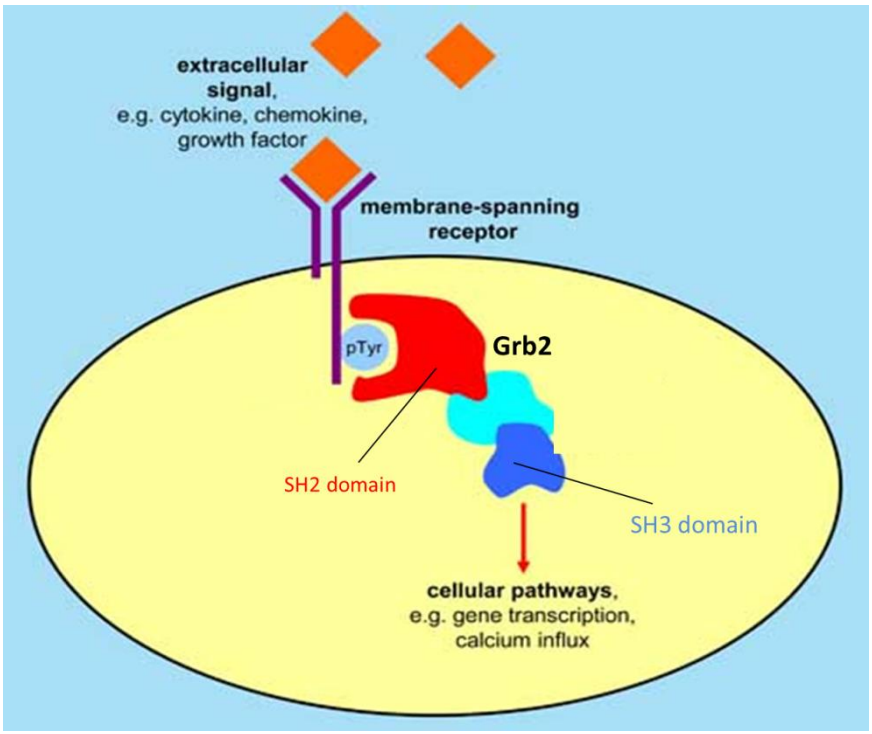


**Figure 3.3**. The signal transduction pathway leading from an extracellular signal to a cellular response. Upon growth factor binding, membrane receptor tyrosines are phosphorylated and recognized by Grb2 SH2 domains. Then, Grb2 is activated, and can move on to activate downstream targets (Adapted from "Tyrosine Phosphorylation and SH2 domains." *Mabtag antibodies.* n.d., Web. 4/20/2015)

Let's now look at how the different elements in this system, the protein Grb2 and the two protein domains, are encoded in the genome and expressed. Grb2 is encoded by the molecular gene *GRB2* in humans. Through the processes of transcription of the molecular gene into RNA and the subsequent translation of the RNA into a protein product, a functional Grb2 second messenger is produced. But there is an interesting process that takes place between transcription and translation that is very relevant to our discussion: splicing. This is a process by which pieces of the transcribed mRNA are removed *before* the ultimate translation into protein happens. In fact, more than 95% of genes in humans are thought to undergo *alternative* splicing, in which differential splicing reactions produce many distinct protein products, called *isoforms*, from the *same* molecular gene. Alternative splicing can produce thousands of different isoforms from a

single molecular gene, all with distinct (but usually related) functions in the cell. As such, the process is often characterized as a way for eukaryotes to produce amazing functional variety from a limited number of molecular genes. GRB2 does not escape the alternative splicing process, which produces a number of protein isoforms in addition to the Grb2 protein. One such isoform is Grb3-3, which is completely missing its SH2 domain, as the SH2 domain-coding fragment is spliced out of the corresponding mRNA. Lacking SH2, Grb3-3 cannot bind the RTK, and thus has a completely different function within the cell: instead of relaying growth signals in the cell, it is responsible for a part of the signaling pathway leading to cell suicide, or apoptosis. Again, since the transcription of GRB2 ultimately results in the production of two functionally different proteins, this molecular gene does *not* have a consistent phenotypic effect.

No matter how the GRB2 domain is alternatively spliced, however, the domain-coding fragments of DNA, SH2 and SH3, *do* retain consistent function in *every* isoform. This is because the splicing boundaries in the mRNA occur in the regions *between* protein domains. Splicing boundaries are almost exclusively located between domains because protein domains are the functional units of proteins, the parts of the protein that are doing the actual work in the reactions or interactions proteins catalyze. If a splicing boundary was to occur *within* a protein domain, it would effectively render the domain nonfunctional. So in Grb3-3, the precise molecular function of SH3 is *still* to bind proline-rich regions of other proteins, albeit not SOS in this case. The SH2 domain is completely absent, meaning it has no phenotypic effect on this particular interactor. The domain-coding fragments for SH2 and SH3 have consistent phenotypic effects, then, whether they appear in Grb2 or Grb3-3.

Of course, just about every rule in biology has its exceptions. There are some instances in which single splicing boundaries appear within protein domains *and* cases when a region of the

protein domain itself is spliced out. But even in these rare cases, the phenotypic effect of the protein-coding fragment is still consistent. First consider a case in which there is a single splicing boundary within the SH2 domain on GRB2. In all likelihood, getting rid of a part of the SH2 domain would render the domain nonfunctional, instead of bestowing some new function upon the domain. The result would be equivalent to getting rid of the domain entirely: the phenotypic effect of the domain-coding fragment is consistently a single effect or no effect at all. Alternatively, a few cases have been described in which small fragments completely within domain-coding fragments have been spliced out of the mRNA. But in all these cases, the domain retains the same function, while the *ability* of the domain to perform the function changes. Consider one example: the protein *Piccolo*, which acts in the pathways of neurotransmitter release, contains a protein domain known as $C_2$, which functions exclusively to bind $Ca^{2+}$ ions. In the mRNA that codes for this protein, the domain-coding mRNA fragment is alternatively spliced into two protein isoforms by the differential removal of a short 27 nucleotide fragment of the mRNA. The $C_2$ domain that has undergone this excision event does not acquire a different function, but rather its *affinity* for $Ca^{2+}$ ions is significantly increased (Garcia *et al* 2003). This shows that even in the rare cases of alternative splicing *within* a domain-coding fragment, the molecular effect of the fragment is effectively preserved.

A second but much rarer form of posttranscriptional editing that takes place in eukaryotic cells is RNA editing (Nishikura 2010). In this process, certain ribonucleotide in mRNA can be altered by enzymes that convert one ribonucleotide directly into another ribonucleotide. The most common change is made by a set of enzymes called *deaminases*, which remove an amino group from a specific adenosine (A) or cytosines (C) ribonucleotides in mRNA and replace it with oxygen, forming ribonucleotides called inosine (I) or guanosine (G) respectively. As a

result, the mRNA sequence corresponding to a gene or protein-coding fragment can be altered without ever changing the DNA sequence! Sometimes, these RNA editing events can introduce premature *stop codons*, which are sequences of three nucleotides that are read by the translational machinery as a signal to stop translation and terminate protein synthesis. In fact, the RNA editing process was discovered when scientists noticed that two different proteins, ApoB100 and ApoB48 are encoded by the *same* molecular gene (Powell *et al* 1987). An RNA editing event that happens exclusively in the tissue of the small intestine produces a stop codon in the mRNA transcript, producing the truncated ApoB48 instead of ApoB100. In the liver, no RNA editing takes place, so the full-length ApoB100 is produced. These two different proteins, ApoB48 and ApoB100, have distinct functions in these distinct tissues; for instance, the premature stop coding results in truncation the domains allowing ApoB100 to associate with the protein LDR, so this cannot happen in ApoB48. Again, we see that the molecular gene that codes for both ApoB48 and ApoB100 does not have a consistent phenotypic effect, as functionally distinct and tissue-specific isoforms are created by RNA editing. However, when RNA editing occurs within a domain-coding fragment, the effect is the same as when alternative splicing occurs within the fragment: a change in the *ability* of the domain to perform a particular function, but no change in the function itself. For instance, a well-documented case of RNA editing within the coding region of a gene occurs in the expression of different glutamate receptors that work to transmit action potentials between neurons (Seeburg and Hartner 2003). In the mRNA transcript of the glutamate receptor gene, several sites of deamination can lead to changes in the actual amino acid sequence of the *transmembrane* protein domain, which spans the cell membrane and acts as an ion channel to selectively let different ions pass in or out of the cell upon glutamate binding. The RNA editing can result in a different level of permeability for

the $Ca^{2+}$ ion, for instance. The function of the domain-coding fragment corresponding to this transmembrane domain of the glutamate receptor thus remains consistent, no matter which RNA editing processes take place in the particular cell environment.

Due to both alternative splicing and RNA editing, the phenotypic effects of molecular genes on low-level interactors are not consistent. Posttranscriptional editing processes do not interfere with the consistency of the phenotypic effect of domain-coding fragments on protein interactors, however, giving domain-coding fragments clear phenotypic effects. These fragments are also small enough to form lineages that may be differentially selected before being broken up by crossing over; such a small fragment very rarely sees crossing over break up the lineage. Clearly, domain-coding fragments also influence the probability that they are copied, by exhibiting consistent phenotypic power on their interactors. We have identified the active replicators in the coding regions of sexually reproducing organisms.

### 4.2. Domain-coding fragments are differentially selected through a hierarchy of interactors

Our next task is to use the replicator/interactor distinction and the identity of the active replicators in the coding sequences to explain how complex adaptation in sexually reproducing organisms can come about through differential selection of these active replicators. This story will also allow us to answer the question about *who benefits* from some complex adaptation. To do this, I will argue that we must first shift our focus from explaining high-level adaptations to explaining how molecular features which are the *basis* for complex adaptation come about. This will involve an approach in which we treat parts of an organism as a hierarchy of cooperating interactors.

To see how complex adaptations that we observe at the level of the organism come about, we must start small, at the molecular level, explaining how the features of the fundamental interactors appear. These features have direct one to one correspondence with certain active replicators, as we have seen in the example of cell signaling we went through; ability to bind phosphorylated tyrosine residues corresponds to the SH2 domain, and the ability to bind proline-rich regions of proteins (such as SOS) corresponds to the SH3 domain. Let's stick with the SH2 domain and ability to bind phosphorylated tyrosines as an example and explain the cumulative selection that led directly to this feature. At one specific locus in the genome, different versions of the SH2 domain-coding fragment are present in different individuals in a population. These versions of the SH2 domain-coding fragment produce not only Grb2 proteins with differing affinities for binding phosphorylated tyrosine residues, but also other isoforms of proteins with different binding affinities through alternative splicing. These isoforms may be participating in a variety of cellular processes; as we saw, Grb2 is important in growth signal transduction while Grb3-3 is involved in apoptosis signaling. But in every single alternatively spliced protein, we see that the phenotypic effect of the SH2 domain-coding fragment is important and beneficial for the functions of that protein and the cellular processes that protein is involved in. Early in the molecular evolution of the first[3] ever SH2 domain ever to appear in the genome, it becomes relatively straightforward to imagine a scenario in which small modifications to the SH2 replicator would increase the binding affinity of the SH2 domain to phosphotyrosine residues, making the protein interactors with the small modification in phenotypic effect perform 'better' in the context of their cellular processes than the proteins in the organisms without the

[3] I say *first* because there are currently hundreds of SH2 domain-coding fragments scattered throughout the human genome, which are parts of hundreds of different molecular genes. These many SH2 domain-coding fragments are not the product of independent, convergent evolution, but rather became prevalent in the genome through gene duplication events following the evolution of some initial SH2 domain-coding fragment. It is the cumulative selection of this *original* SH2 domain that I am describing here.

modification (I will get back to what it means for certain sets of proteins to function 'better' than others in a moment). Eventually, the organisms (or *vehicles*) with the isoforms that work better will leave more offspring than the organisms with the isoforms which do not work as well, leading to the differential selection of the SH2 domain-coding fragment. We thus see how a clear selection pressure towards high binding affinity for phosphorylated tyrosine residues develops, leading to the appearance of this characteristic of proteins through cumulative selection of small modifications.

A large gap in this explanation remains, however, since it might be difficult to imagine how tiny changes in the affinity of a set of isoforms to phosphorylated tyrosine residues might result in the differential reproduction by the organism. It is to bridge this gap that it is useful to think of a hierarchy of cooperating interactors within the organism. Let's begin on the fundamental level of interactors: proteins. As interactors, proteins interact with the surrounding environment as cohesive wholes, resulting in the differential selection of the active replicators which provide the phenotypic effects of the protein domains. Now, the nature of the interaction between proteins and the environment is very different from the interaction and environment of the fundamental interactors in asexually reproducing organisms like bacteria. Remember that a bacterial interactor interacts with an external world filled with mostly nonliving entities, with the way the interactor responds to different stresses and stimuli affecting whether it is successful in surviving and gathering the resources needed to replicate its bacterial chromosome. The environment of proteins, however, is made almost exclusively of *other interactors* that share the same vehicle. Proteins *must* cooperate with the interactors built by other active replicators in order to build higher-level interactors and the vehicles that eventually determine the fate of the active replicators in the germ-line. Successful proteins, then, are the ones that *cooperate well*

with other proteins, and the active replicators that are most successful are the ones whose domains help their proteins cooperate. The environmental interaction for interactors in sexually reproducing organisms is all about cooperation.

Cooperation between interactors at different levels allows us to clearly see how a small modification of binding affinity in the SH2 domain can result in a selective advantage. The set of isoforms containing the SH2 domain that works 'better' or is more successful is the set that cooperates effectively with other proteins to improve some cellular process in a higher level interactor, the *cell*, which in turn cooperates better with surrounding cells to produce an organism with slightly higher fitness. In the early days of SH2 domain-coding fragment evolution, a higher affinity for phosphorylated tyrosine residues would allow the GRB2 second messenger protein to be better able to find its activated RTK and bind that receptor for a longer period of time, allowing for more robust activation of these second messenger proteins every time a growth signal is received. Grb2 thus cooperates better with the other proteins in the signal transduction cascade, making the transduction cascade more sensitive to an incoming signal. Slightly increased sensitivity to incoming growth signals would allow a higher-level interactor, the cell, to cooperate better with other interactors at the same level, or other cells. Increased sensitivity to growth signals allow cells to accurately direct and coordinate growth during development and promote cell differentiation in specific areas of the adult organism as well. Again, the cells are interacting not with the outside world but with an environment composed of other *interactors*, so the best metaphor for this interaction is cooperation, how well the cell cooperates with other cells to produce a successful organism determines the fate of all the replicators in the germ line of that organism. Finally, cooperation between cells throughout an organism ultimately determines if that organism will reproduce and ensures that the germ-line

lineage of SH2 domain-coding fragments is replicated. Since *all* the cells in those organisms with SH2 domains with slightly higher binding affinities for phosphorylated tyrosine residues will cooperate better, this may very well result in an overall growth that is significantly more coordinated or even a faster growth in a juvenile organism. Organisms that are better at coordinating growth will have a selective advantage over those organisms that are slightly worse at performing this important function.

The ability to bind phosphorylated tyrosine residues with high affinity might not qualify as a complex adaptation, but it is nevertheless an amazing feature of an organism whose appearance we can now understand and appreciate using the tools of the replicator/interactor distinction resting on the consistent phenotypic effect of active replicators and a cooperating hierarchy of interactors. Of course, the story I told about the cellular processes and communication benefitted by higher SH2 binding affinities is merely informed speculation. In all likelihood, the perfection of the SH2 domain's phenotypic effect took place many millions of years ago, in organisms that were ancestors to the wide variety of organisms that contain SH2 domains today. But something *like* the story I described takes place to cause the cumulative selection of not only SH2 domain-coding fragments, but the hundreds of thousands of domain-coding fragments present in the genomes of sexually reproducing organisms. The hope is that my framework will make it easy to *imagine* how features of a protein could appear, so we can wrap our head around the incredible designs we see everywhere in the molecular machines that are the building blocks of life.

Notice that another benefit of explaining features of fundamental interactors is that the features have direct correspondence to the active replicators (in fact, this is one of the characteristics of a fundamental interactor). As a result, it is very clear what that feature *is good*

*for*, or which entity benefits ultimately from that feature: it's a domain-coding fragment. The whole upwards cascade of cooperating replicators that use the increased affinity to phosphorylated tyrosine residues increases the probability that the SH2 domain-coding fragment is replicated *along with* the hundreds of thousands of other replicators present in that particular organism, but remember that in the next generation, the SH2 domain-coding fragment is going to be paired with an entirely new set of active replicators due to crossing over. Still, since the effect of the domain-coding fragment is consistent, as organisms with successful SH2 domains will have a set of isoforms with increased affinity for phosphorylated tyrosine residues, so *in the long run* it is *only* the SH2 domain-coding fragment that benefits from this feature.

On the molecular level in sexually reproducing organism, then, the replicator/interactor distinction does a great job at explaining the appearance of precise molecular machinery and who benefits from the precision. What follows directly from this is the ability to begin to understand features at the level of the whole sexually reproducing organism. We have already seen this play out in our story of signal transduction cascades, in which the SH2 domain played an important role in overall growth and development of an organism. By taking this bottom up approach to looking at the evolution of features at the level of the organism, it becomes clear that it is impossible to directly identify a single active replicator or even a precise set of active replicators whose differential selection is directly responsible for an organism-level feature. If we begin by asking what a trait of a higher-level interactor is good for, or how it came to be, we will get lost trying to explain how the many replicators that are responsible for parts of that trait are differentially selected for that particular trait *and* the many other traits that their consistent phenotypic effects play a role in promoting. Much of the difficulty is simply a result of the lack of direct one-to-one correspondence between active replicator and function on the level of the

organism. In other words, asking about the origin of a high-level trait *first* is simply asking the wrong question. Instead, I am advocating for a bottom-up approach where we ask how the traits of fundamental interactors come about first. By appealing to the differential selection of active replicators, it becomes clear how the features of fundamental interactors come about, which can, in turn, help us understand how the hierarchy of interactors cooperates to produce the complex adaptations we see in whole organisms.

## 5. Regulatory elements are the active replicators in sexual genomes that mediate phenotypic changes by modifying gene expression

The coding regions of the genome contain the active replicators whose differential selection is the basis for the functional characteristics of proteins. Thus, analyzing the differential selection of domain-coding fragments is sufficient for understanding the appearance of characteristics of protein. But merely understanding the functional characteristics of proteins is not nearly enough to comprehend how proteins can cooperate to produce higher-level interactors, or how natural selection could design these higher-level cooperative processes. This is because how well proteins cooperate is determined not only by their specific molecular functions, but also by the expression patterns of genes in various cellular contexts. The precise regulation of the expression of the 20,000 genes in a human genome into hundreds of thousands of proteins is an incredible feature of a higher-level interactor itself, the cell, which requires an origin explanation. Equally amazing are the changes in gene expression that lead to incredible changes in the morphology on the level of the organisms in many species: just think of the metamorphosis of a caterpillar into a butterfly or the many stages in the complex multi-host life cycle of a trematode worm. Part of gene expression certainly depends on the functional

characteristics of proteins. For instance, there are protein domains that allow proteins called transcription factors to bind sequences of DNA and block or stimulate transcription. But notice that in this case, these transcription factors are not cooperating with other proteins. Rather, they are cooperating with *regulatory elements* which are active replicators in their own right. In this final section, then, we will survey the different types of regulatory elements in the eukaryotic genome and see why they should be considered active replicators along with domain-coding fragments and transposons. I will also show how appealing to the differential selection of regulatory elements is necessary for explaining the evolutionary origin of the many features of higher-level interactors that involve responses to the environment.

### 5.1. Regulatory elements

There are two major types of DNA sequences which I refer to as 'regulatory elements' here. One is the group of DNA sequences that help influence the expression of genes by binding proteins called transcription factors. The other is the group of DNA sequences that are transcribed into a special kind of RNA called microRNA (miRNA) but are *not* translated into proteins. In turn, these miRNAs act to specifically influence gene expression by an interesting mechanism called RNA interference. But let's begin by looking at the more well-known cases of regulatory elements that recruit and bind proteins to affect levels of transcription. These regulatory sequences can range anywhere from several nucleotides to many hundred nucleotides in length, but they are always short enough for relatively small selection pressures to cause cumulative change in the sequence lineage despite crossing over. The phenotypic effect of these sequences is also consistent simply because they interact with a certain protein domain with high affinity, qualifying these fragments as active replicators in Dawkins's framework.

Let's take a look at a simple example that illustrates how the differential selection of these sequences plays an integral role in explaining aspects of gene expression. One highly conserved regulatory element which is found in the promoter sequence upstream of the transcription start site of almost *every* molecular gene in a eukaryotic genome is called the TATA box. The TATA box is a sequence of just 6 nucleotides (5'-TATAAA) that recruits and binds the transcription factor TATA-binding protein (TBP) (Cox *et al* 2012). Upon the binding of TBP, the DNA around the TATA-box is severely distorted and partially unwound, which is an essential step in order for transcription of the upstream gene to occur (Figure 4). In this case, it is relatively straightforward to imagine how cumulative selection resulted in a conserved sequence element with a high affinity for a DNA-distorting protein. Organisms with versions of the TATA box that had higher affinities for the TBP would probably have a much easier time transcribing that particular gene, since the TBP-DNA interaction does a lot of the work involved in initially separating the two DNA strands that is necessary for transcription to occur. Through duplications of the promoter to other regions of the genome or through convergent evolution (which is likely since this sequence is so short), most molecular genes in eukaryotes became dependent on an upstream TATA box for transcription. Thus, a story about cumulative selection of regulatory active replicators like the TATA is an essential part of explaining how a process like transcription came about.

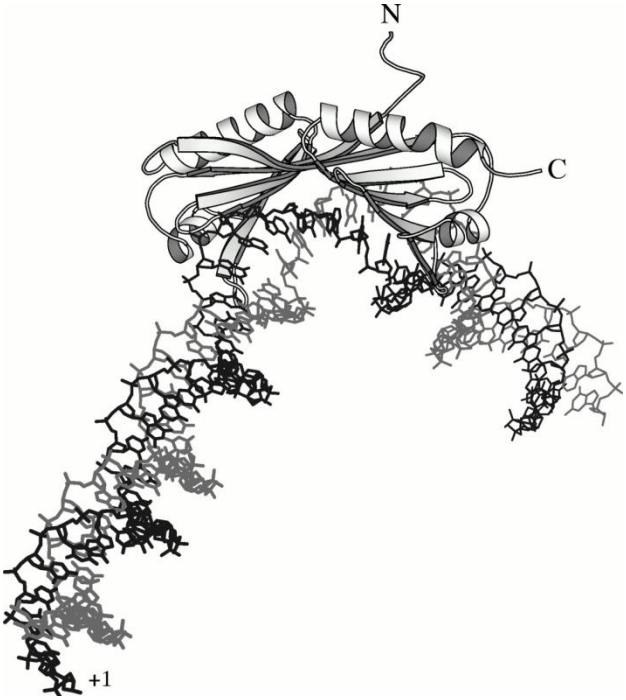


**Figure 4.** Interaction between TBP (top) and the TATA box (near the center of the DNA strand, below). Note how the TBP kinks the DNA, forcing partial unwinding that is essential to transcription initiation (From Mirkin 2015).

The differential selection of DNA-binding elements will even add explanatory power to the story about the differential selection of the SH2 domain-coding fragments we encountered in the previous section. Note that one of the steps in the process of cell growth signaling that was not explained in great detail was how the signaling cascade including Grb2 as a second messenger results in cell growth. It turns out that the signaling cascade eventually phosphorylates specific transcription factors that have a high affinity for DNA sequences called *enhancers.* Enhancers are regulatory elements which are usually 50-100 base pairs long but located hundreds or even thousands of bases away from a molecular gene, and have high binding affinities for a specific transcription factors. The binding of transcription factors to enhancers is not *essential* for transcription to occur unlike the binding of transcription factors in the promoter region of the molecular gene; low, basal levels of transcription can still occur in the absence of

this interaction. However, when the enhancer binds its transcription factor, transcription of the corresponding gene is greatly 'enhanced'. In the case of growth signaling, the enhancers specific for genes involved in growth recognize *phosphorylated* transcription factors. Since the transcription factors can only be phosphorylated after the cell receives a signal which is passed down via the Grb2-mediated cascade, the enhancer-transcription factor interaction only increases transcription of growth genes after a signal is received. So the protein interactors in the signal transduction cascade must cooperate not only with other protein fundamental interactors, but also the fundamental interactors of regulatory elements. Notice that in the case of these DNA-binding regulatory elements, the replicator and the fundamental interactor are the same entity. How successful these regulatory sequences are in interacting and cooperating with proteins in a way that is beneficial to higher-level interactors will determine how likely the regulatory sequences in the germ-line of the vehicle are to be transmitted to the next generation.

The other group of regulatory elements, the sequences coding for non-coding miRNAs, do have separate interactors. Again, these sequences are short enough that their cumulative selection is not affected substantially by crossing over, and they bestow very consistent phenotypic effects to their interactors, the miRNAs. The sequences responsible for the miRNAs are first transcribed into an RNA molecule that folds back on itself, forming a hairpin. This hairpin is then cleaved and processed by a few different proteins and eventually all that remains is a short 21-23 nucleotide long miRNA sequence that associates with a protein complex called RNA-induced silencing complex (RISC). Once this complex and the RNA are assembled, the precise regulatory mechanism called RNA silencing can take place. The short miRNA functions as a *guide sequence* and binds tightly with a complementary mRNA sequence somewhere in the nuclear environment. Then, proteins in the RISC complex quickly chop up this target mRNA.

The same RISC/miRNA then moves on to target additional mRNAs, effectively removing many target mRNAs from the cell and specifically preventing a protein or group of isoforms from being produced. Here, the consistent phenotypic effect we see for the miRNA coding sequences is the tight binding to the target mRNA that allows the RISC complex to find its silencing target. Again, it is not too difficult to see how this kind of regulatory mechanism could allow coordination and cooperation on a cellular level. For instance, there might be selection pressure to develop a miRNA that is complementary to a gene involved in cell differentiation which could then be expressed once the cell receives a cue to stop dividing. This would allow for better coordination and cooperation among the cells in an organism.

Finally, it is worth mentioning that there are also a few kinds of noncoding RNAs (ncRNA, RNAs that are not translated but have some molecular function) that associate with proteins to form *ribonucleoproteins* but do not function in gene regulation and expression. One example of an important ribonucleoprotein is the most abundant protein complex in cells, the ribosome, which is actually 60% RNA. In the ribosome, RNA has both structural and catalytic roles in protein synthesis (it is commonly referred to as a *ribozyme*), with different parts of the RNA carrying out different specific functions. We can call the parts of the RNA that carry out these functions "RNA domains", and the fragments of DNA that code for these domains RNA-domain-coding fragments. As such, the evolutionary dynamics of these regions will be completely analogous to the dynamics of the domain-coding fragments described in the previous section, so there is no need to examine this case here in detail.

By allowing genes to be differentially expressed in response to certain environmental cues, regulatory elements are a key component of the coordination that allows low-level interactors to cooperate within a higher-level organism. Another key component of this

coordination is another set of ribonucleoproteins, the *splicing factors*, which carry out the splicing reactions that lead to a range of processed mRNA transcripts of nearly every eukaryotic gene. The regulation or splicing factors by regulatory elements and their corresponding transcription factors determines which set of isoforms is produced from a certain mRNA molecule. So it is the cooperation between the active replicators in the regulatory elements, the domain-coding fragments that are part of transcription factors, and the sequences coding for the ncRNAs present in splicing factors that is ultimately responsible for the differential expression of molecular genes.

### 5.2. Differential selection of regulatory elements plays a major role in explaining both phenotypic changes on the level of the whole organism and changes at the molecular level.

Much of the criticism that has been leveled at gene selectionism in recent years has focused on the inability of the gene's eye view to account for phenotypic features that are based in large-scale changes in gene *expression* (Dobbs 2013). For instance, it may come as a surprise that peaceful, solitary grasshoppers and destructive, swarming locusts are actually the same species of insect, with the exact same genome. When grasshoppers are exposed to conditions of food shortage and drought, solitary individuals begin to cluster together in groups. Hoppers sense this increased population density through stimulation of their hind legs, triggering their remarkable transformation into locusts (Rogers *et al* 2003). The increased stimulation results in the release of serotonin from the grasshopper brain, which in turn results in the differential expression of genes that cause a number of physical and behavioral changes: the body color of the animal becomes markedly darker, the flying muscles strengthen, metabolic rate increases, and the locusts travel in groups devouring anything green in their path. Now, cooperation

between hierarchies of interactors corresponding to the coding regions of the genome may explain the physical features of both the grasshopper and locust. However, those who object to gene selectionism argue that evolution of molecular genes does not explain how the *changes* that take place when a grasshopper becomes a locust could evolve.

Surprisingly, this objection simply misunderstands what gene selectionism means by the word 'gene'. Dawkins was very clear in *The Extended Phenotype* that *genes* should be regarded as any collection of DNA fragments that are responsible for a difference in phenotype, regardless of whether those fragments are located within the coding sequence of the genome. So the gene responsible for the differences between different versions of locust transformation is located in the noncoding regions of the genome. But notice, again, that these Mendelian genes are unlikely to form lineages, and it is thus difficult to see how cumulative selection to this group of genes could take place. Replicator selectionism, on the other hand, handles the evolution of the locust transformation brilliantly. We can easily imagine how such cumulative selection might take place by identifying specific regulatory elements as the replicators responsible for molecular effects that play a role in locust transformation. For instance, enhancer sequences near the molecular genes whose expression results in the locust phenotype might have been differentially selected to preferentially bind a transcription factor bound to serotonin over the transcription factor alone. This way, only when serotonin concentrations are high and the transcription factors bound to this neurotransmitter can the transformation genes be transcribed. The grasshoppers with regulatory elements that contain small modifications that allow them to better distinguish between transcription factor and transcription factor + serotonin, then, would be selected over the hoppers without this modification, since the locust phase is a great way to find food but *only* under stressful conditions. Of course, the underlying mechanisms are probably much more

complicated than what I just described, but again, the point is that we can *imagine* how selection pressures at specific DNA sequences can lead to the evolution of these phenotypic changes.

By appealing to the differential selection of regulatory elements, we can explain the evolutionary origins of not only the changes in gene expression due to the environment, but also the differential expression of molecular genes in different tissues that lead to specialization of individual body parts. The regulatory elements controlling the expression of genes responsible for cell differentiation, for instance, evolved by cumulative natural selection to bind specific transcription factors that control this differentiation process. Now, some have taken a very different approach to modeling the complicated expression patterns of molecular genes: treating active replicators as *strategic entities.* In *The Social Gene* (1997), David Haig argues that by anthropomorphizing replicators and treating them as players in an evolutionary game, we can see how evolutionary stable strategies of gene expression for different environments can be selected from a set of alternative strategies by cumulative natural selection. In turn, these strategies can help explain why replicators 'behave' the way they do in different contexts.

Notice, however, that when we limit replicators to fragments of DNA that have a consistent phenotypic effect we no longer *need* to use shorthand and treat the fragments as strategic entities. Active replicators no longer have complicated behavioral characteristics which change in different contexts; rather, their behavior is simple and straightforward due to their consistent phenotypic effect, while it is the complicated collaboration between the interactors that results in cumulative selection of the replicators toward or away from their specific function. Now, it may still be *useful* to treat molecular genes as strategic entities if we want to explain the evolutionary dynamics of genes, just as it is useful to treat organisms as players in evolutionary games. We might even treat other low-level interactors as strategists, such as proteins and cells,

to help visualize what cooperative strategies they employ in order to achieve multilevel cooperation. However, we must keep in mind that in these cases, the interactors and genes are *not* the fundamental evolutionary units, the units of selection; we have seen that Haig and company are mistaken in regarding genes as active replicators. Rather, it is the collaboration between many regulatory elements, transcription factors, and splicing factors that confer the complexity that *allows* genes and their corresponding interactors to behave like strategic entities.

## Concluding remarks

When we look at the incredible design features of organisms, one of the first questions we seek to answer is how those design features evolved by natural selection. Although it certainly is tempting to begin our evolutionary explanations with these high-level traits, I have argued here that we ought to be a bit more careful if we want our explanations to include all the mechanistic details which we now understand thanks to the molecular biology. Taking a bottom-up approach and *first* explaining the evolutionary origins of the characteristics of fundamental interactors allows us to see exactly which individual active replicators are differentially selected through cumulative natural selection to produce those characteristics. *Then*, through a hierarchy of cooperating interactors, we can see how the features of cells and vehicles come to be, whether these features are physical characteristics whose evolution are based in coding regions or *changes* in physical characteristics which are based in both the coding regions and regulatory elements.

In order to identify the fundamental replicators whose design features we should seek to explain in the first place, we must begin by identifying the active replicators. After all, the fundamental interactors are the entities which carry the consistent phenotypic effects of the

active replicators; if we know which DNA fragments are the active replicators, we simply look at their consistent phenotypic effect to identify the fundamental interactor. Importantly, under *no* circumstances are molecular or Mendelian genes active replicators. I have argued that in order for the replicator/interactor distinction to be useful in explaining design across species, replicators must have consistent phenotypic effects *and* longevity. Neither conception of the gene fits both of these requirements. Of course, the gene is still an incredibly useful concept in biological explanations, but we should abandon the idea that genes are the basic units of selection.

There is one type of feature of higher eukaryotic organisms that my framework has yet to explain, however: the appearance of traditions or culture. The transmission of culture from parent to offspring can occur through non-genetic means, with evolutionary dynamics completely independent of the DNA-based replicators (Avital and Jablonka 2005). Could the same replicator selectionism framework developed here be applied to the evolution of units of culture, or *memes* (Dawkins 1976)? Perhaps, but this might require us to accept that active replicators are made of pieces of *information* rather than physical genetic elements. It is difficult to imagine how the precise identification of active replicators made of units of information might go, a process which we have seen is the first essential step in replicator selectionism. For instance, it will be a challenge to determine if an information-based replicator has a consistent phenotypic effect.

So the replicator/interactor distinction may not be a useful framework for understanding Darwinian cultural evolution. Indeed, the replication of certain structures, physical or informational, may not even be a *necessary* feature of evolution; Peter Godfrey-Smith has come up with interesting thought experiments in which we get evolution by natural selection without

replicators (2000, 2011). Heredity doesn't necessarily have to proceed through replication of certain structures, if it is achieved by some other means. But even if the replicator/interactor distinction is not an essential feature of evolution, it *is* a feature of the evolutionary change based in genetic replicators that brings about the vast majority of design we see in nature. And while Darwin's theory of evolution by natural selection may very well be a satisfying explanation in itself for the appearance of these incredible designs for most people, for those of us who long for a mechanistic explanation that allows a complete picture of evolution including the molecular details of heredity, identification of active replicators is the best place to start.

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