Evolution of metamorphosis: role of environment on expression of mutant nuclear receptors and other signal-transduction proteins

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Synopsis Various lipophilic signals, including ecdysone, retinoic acid, estradiol, cortisol, testosterone, and progesterone, act through nuclear receptors, a large group of transcription factors that regulate differentiation and development, which are central to metamorphosis. Here, we focus on environmental factors (for example climate and chemicals) in the evolution of nuclear receptors and other signal-transduction proteins that interact with heat-shock protein 90 (Hsp90), a chaperone that promotes the proper folding and trafficking in cells of proteins. Hsp90 also promotes functional folding of some mutant signal proteins, which would be otherwise destabilized. Stress diverts Hsp90 from stabilizing mutant signal-transduction proteins and toward promoting proper folding of stress-damaged proteins and preventing the aggregation of denatured proteins. Reduced Hsp90 levels allow expression of cryptic mutations in signal-transduction proteins and new developmental patterns. Thus, environmental stress in the form of extreme climate can influence the evolution of metamorphosis. We discuss how extreme cooling called "Snowball Earth," which occurred in the late Proterozoic, diverted Hsp90 from chaperoning signal-transduction proteins. As a result, pre-existing mutant signal-transduction proteins were expressed in animals. Some mutations were selectively advantageous in animals that are seen in the Cambrian, when diverse pathways for metamorphosis in metazoans first appear in the fossil record. Other environmental factors, such as biological chemicals (for example the antibiotic geldanamycin) can reduce the levels of active Hsp90 providing another mechanism for the emergence of mutant signaling pathways.

Introduction

Scientific studies of metamorphosis can be traced back to Aristotle. Although Aristotle did not use the term metamorphosis, his scientific interests included observation of changes in the form of insects, a process that we now call metamorphosis (Llyod 1996). Moreover, life-changing transitions were an important part of the non-scientific Greek culture, as described in their myths. In this regard, we owe much to the Roman poet Ovid, who collected various Greek myths into the poem "Metamorphosis" (Martin 2004). This work stimulated the imaginations of many poets and philosophers for almost 2000 years. In these myths, metamorphosis could involve such things as humans changing into animals or inanimate objects or animals changing into humans. In one story, Ovid describes the transformation of Daphne into a tree to escape Apollo. In another story, Ovid describes how Bacchus gave King Midas the ability to transform whatever he touched into gold.

Biologists have adapted metamorphosis to describe the various stages of animal development (Gilbert and others 1996; Thummel 1996; Power and others 2001; Youson and Sower 2001; Truman and Riddiford 2002; Heyland and others 2005; Tata 2006) such as the change of a larva into a pupa and the emergence of the adult at the end of the pupal stage. This process describes how insects, mollusks, and some fish develop. Frogs provide another example of metamorphosis. A tadpole emerges from the egg; the tadpole lives in the water, breathes with gills, and has a tail. As the tadpole grows, it develops lungs and legs, and the gills and tail are absorbed into the body. Finally, the frog leaves the water and lives mainly on land.

Global transformations from 1 life stage to another in metamorphosis involve changes in cells as they differentiate to form new organs and the loss of old organs through cell death. This process is morphogenesis, which is discussed along with metamorphosis in the present paper.

Hormones regulate metamorphosis

A variety of hormones regulate morphogenesis and metamorphosis in insects, crustaceans, echinoderms,

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fish, and amphibians (Gilbert and others 1996; Thummel 1996; Power and others 2001; Youson and Sower 2001; Truman and Riddiford 2002; Heyland and others 2005; Tata 2006). Two lipophilic hormones that are important signals for metamorphosis are ecdysone and thyroid hormone. Ecdysone is a molting hormone for arthropods, which include insects and crustaceans (Thummel 1996; Truman and Riddiford 2002; Gilbert and others 2002). Thyroid hormone is a signal for metamorphosis in many deuterostomes, including echinoderms, lamprey, and amphibians (Power and others 2001; Youson and Sower 2001; Heyland and others 2005; Tata 2006). Retinoic acid is another important regulator of morphogenesis in deuterostomes (Gudas 1994; Mark and others 2006).

The actions of these 3 hormones are mediated by the ecdysone receptor (EcR), thyroid hormone receptor (TR), and retinoic acid receptor (RAR), which belong to the nuclear receptor family (Laudet 1997; Escriva and others 2000; Olefsky 2001), a large group of transcription factors. This family also contains receptors for estrogens (ER), androgens (AR), glucocorticoids (GR), mineralocorticoids (MR), and progestins (PR) (Laudet 1997; Baker 1997, 2003). These steroids influence many aspects of morphogenesis in vertebrates. Moreover, some steroid-regulated pathways can cross-talk with pathways that are regulated by thyroid hormone and retinoic acid (Zhang and others 1996; Crespi and Denver 2005). This increases the combinatorial possibilities for differentiation and development during metamorphosis in deuterostomes.

Nuclear receptors are restricted to metazoans

A question relevant to the origin of the various pathways found in metamorphosis is when did TR, ER, RAR, steroid receptors, and other nuclear receptors evolve? Analysis of various eukaryotic genomes has revealed that nuclear receptors are restricted to metazoans (Laudet 1997; Escriva and others 2000; Thornton 2001; Baker 2005). Nuclear receptors appear to have evolved by series of gene duplications, followed by functional divergence of the duplicated gene (Laudet 1997; Baker 1997, 2002, 2003; Escriva and others 2000; Thornton 2001; Bertrand and others 2004).

What influenced the evolution of the ER, TR, RAR, and steroid receptors from ancestral nuclear receptors to yield receptors that regulated morphogenesis and metamorphosis? The evolution of these receptors in metazoans appears to have occurred when new and more complex metazoans occupied the biosphere (Knoll and Carroll 1999; Bertrand and others 2004). This resulted in more complex interactions between animals due to factors such as competition for food and the emergence of predators (Knoll and Carroll 1999; Peterson and Butterfield 2005; Peterson and others 2005). One contribution to survival in this environment was the evolution of new ligand-activated nuclear receptors, which provided additional pathways for adaptive differentiation and development.

In the present paper, we focus on the effect of extreme changes in climate on the evolution of ligand-activated nuclear receptors and other transcription factors that mediate morphogenesis and metamorphosis. Although this occupies most of our discussion of the evolution of metamorphosis, we also consider the role of signals from organisms in the biosphere on the evolution of signal-transduction proteins. The first question is when did these diverse pathways for morphogenesis and metamorphosis evolve in metazoans? That is, when did metazoans evolve?

The Cambrian explosion

It was during the Cambrian explosion, an interval from 543 to 530 million years ago, that the body plans seen in modern animals suddenly appeared in the fossil record (Fortey and others 1997; Valentine and others 1999; Conway Morris 2000; Budd and Jensen 2000; Peterson and others 2005). In geological time, an interval of 13 million years is "a blink of the eye." It is remarkable that ~32 phyla appear to have evolved in such a brief period. The Cambrian provides the first extensive fossil evidence for diverse signaling pathways for morphogenesis and morphogenesis.

The fossil record, however, only informs us of the latest time when these pathways arose. To go back further in time, one uses bioinformatic analyses of "molecular fossils," which are either DNA or amino acid sequences of genes that are selected from cndiarians, protostomes, and deuterostomes. The sequences are collected, aligned, and then analyzed to calculate when they first appeared in metazoans. This analysis relies on a molecular clock for the rate of mutation for each protein (Rodriguez-Trelles and others 2002). A problem is that it is unrealistic to assume a constant molecular clock for mutations in genes over 700 to 1000 million years. To deal with this problem there are sophisticated algorithms that can identify changes in the molecular clock in genes over long intervals and then correct for these changes.

With these corrections, it would appear to be a straightforward computer analysis, to determine when protostomes and deuterostomes evolved. However, different laboratories have reached different conclusions. Indeed, there remains much controversy about

when the various phyla emerged (Aris-Brosou and Yang 2003; Blair and Hedges 2004; Peterson and others 2004; Peterson and Butterfield 2005; Welch and others 2005). Some propose that arthropods and deuterostomes diverged ~1000 million years ago (Blair and Hedges 2004); others predict a more recent divergence from 750 to 570 million years ago (Ayala and others 1998; Bromham and others 1998; Aris-Brosou and Yang 2003; Benton and Ayala 2003; Douzery and others 2004; Peterson and Butterfield 2005; Welch and others 2005).

As will be discussed below, these differences do not affect our suggestions regarding the influence of climatic change on the emergence of diverse signaling pathways for morphogenesis and metamorphosis.

Snowball Earth and the Cambrian explosion

There are 2 extreme glaciations that ended ~670 and 635 million years ago and a third period of cooling that ended ~580 million years ago during the Neoproterozoic (Hoffman and others 1998; Hyde and others 2000; Runnegar 2000; Lubick 2002). The 2 extensive glaciations either froze the entire ocean—Snowball Earth (Hoffman and others 1998)—or most of the ocean—Slushball Earth (Hyde and others 2000; Runnegar 2000; Lubick 2002). Each freezing event was followed by a warm interval that melted the ice. These severe climate intervals have been proposed to have played an important role in the Cambrian explosion (Baker 2006).

One likely outcome of Snowball Earth is that it caused either extinction of many organisms or the severe reduction in the populations of other organisms on Earth. This created population bottlenecks in surviving Neoproterozoic animals, leading to a change in the organisms on Earth during the radiation that repopulated the biosphere, after the glaciers melted (Hoffman and others 1998; Peterson and others 2004, 2005).

We proposed that extreme climatic events also promoted the evolution of new signaling pathways (Baker 2006), some of which are important in morphogenesis and metamorphosis. In this way, Snowball Earth may have been a cauldron for genetic variation and diversification of animal phyla, as seen in the Cambrian. Critical to our hypothesis is the effect of extreme climate on heat-shock proteins, including heat-shock protein 90 (Hsp90), as described below.

Hsp90 stabilizes signal-transduction proteins

Hsp90 is a highly conserved multifunctional protein that is essential for the viability of eukaryotes from yeast to humans (Feder and Hofmann 1999; Rutherford 2003; Sangster and others 2004). Hsp90 acts as part of a complex that includes Hsp70, p23, and other proteins (Pratt and Toft 2003). The Hsp90 complex promotes the proper folding and intracellular location of a variety of signal-transduction proteins that are not functional in the absence of Hsp90. Some of these proteins are ligand-dependent transcription factors, such as nuclear receptors (for example EcR, RAR, AR, ER, GR, MR, PR) and the aryl hydrocarbon receptor; others, such as MyoD and mutated p53, are ligand-independent transcription factors. Hsp90 also stabilizes tyrosine kinases and serine/threonine kinases that act in the mitogen-actived protein pathway (Smith and others 1998; Feder and Hofmann 1999; Mayer and Bukau 1999; Young and others 2001; Picard 2002; Rutherford 2003; Pratt and Toft 2003; Sangster and others 2004). Thus many signal-transduction networks that are important in morphogenesis and metamorphosis depend on Hsp90 for functional integrity.

An important property of Hsp90 is that it also stabilizes mutant transcription factors, which would not fold properly, and thus be destabilized and inactive in the absence of Hsp90. By promoting the proper folding and intracellular localization of mutant signal-transduction proteins, Hsp90 maintains the normal phenotype in the presence of underlying genetic variation, a process called canalization (Waddington 1942; Wilkins 1997).

Snowball Earth interferes with Hsp90 stabilization of mutant transduction proteins

Under stress, Hsp90 is diverted from buffering mutations in signal-transduction proteins and toward its role as a chaperone to promote the proper folding of stress-damaged proteins and to prevent the aggregation of denatured proteins. Reduced levels of Hsp90 allow the expression of cryptic mutations in signal-transduction proteins, leading to new developmental patterns.

This interesting connection between Hsp90 and the evolution of mutant animals was first reported by Rutherford and Lindquist (1998) in a series of experiments with *Drosophila* that were heterozygous for mutant Hsp90. Due to mutant Hsp90, these animals had a variety of developmental abnormalities in wings, eyes, and legs that were due to mutations from several genes. Exposure of *Drosophila* heterozygous for mutant Hsp90 to temperature stress at either 18 or 30°C instead of the normal 25°C revealed cryptic mutations due to diversion of Hsp90 to promote proper folding

of stress-damaged proteins. Thus, moderate temperature fluctuations, which can occur in the wild, will interfere with normal Hsp90 function, allowing expression of hidden genetic variation in wild-type *Drosophila* populations. Importantly, these polygenic mutations could be selected for expression in *Drosophila* with wild-type Hsp90.

Role of Hsp90 in morphogenesis and metamorphosis

The extreme climatic events in the late Proterozoic would affect a wide variety of signal-transduction proteins that are stabilized by Hsp90. Three examples that are relevant to metamorphosis are the evolution of receptors for ecdysone, retinoic acid, and thyroid hormone. These 3 receptors belong to the NR1 subfamily of nuclear receptors. Hsp90 forms complexes with the EcR (Arbeitman and Hogness 2000). Unlike the EcR, RAR is not found in a complex with Hsp90; however, RAR activity depends on Hsp90, which may be due to a requirement for transient binding and stabilization by Hsp90 when RAR folds after translation and is transported into the nucleus (Holley and Yamamoto 1995).

By affecting Hsp90 levels, environmental stress in the form of extreme climate can promote the expression of pre-existing mutants of a variety of signal-transduction proteins, including EcR and RAR. In this way, Snowball Earth promoted diversification of nuclear receptors and other signal-transduction proteins, increasing the combinatorial options for regulating metamorphosis that we see in the diverse animal phyla that appeared in the Cambrian.

Hsp90 does not form stable complexes with human TR

In human cell extracts, TR is found in the nucleus bound to DNA and not in a complex with Hsp90. Soluble complexes of TR with Hsp90 have not been observed (Dalman and others 1990; Privalsky 1991; Pratt and Toft 2003).

The absence of complexes between TR and Hsp90 and its associated proteins is intriguing due to the critical role of thyroid hormone in metamorphosis in deuterostomes. Interestingly, v-erb A, a mutant TR, interacts with Hsp90 (Privalsky 1991), indicating that a closely related protein retains determinants for forming a stable complex with Hsp90. Indeed, Privalsky suggested that the normal TR may form transient complexes with Hsp90 during folding or transport to the nucleus, in which case, TR would resemble RAR (Holley and Yamamoto 1995).

There is evolutionary support for Privalsky's hypothesis from a phylogenetic analysis of TR, which has it clustering with RAR in the NR1 subfamily of nuclear receptors (Baker 1997, 2005; Laudet 1997; Escriva and others 2000; Bertrand and others 2004). CAR, PPARα, and PXR, which also are in the NR1 subfamily, form stable complexes with Hsp90 (http://www.picard.ch/downloads/downloads.htm). We propose that the ancestral NR1 subfamily receptor formed stable complexes with Hsp90 and the TR/RAR ancestor formed either stable or transient complexes with Hsp90. This property was either lost or modified in TR and RAR descendents during the evolution of deuterostomes. Alternatively, the formation of stable complexes between Hsp90 and CAR, PPARα, PXR, EcR, and v-erb A and transient complexes between Hsp90 and human RAR evolved independently from the NR1 ancestor that did not form either transient or stable complexes with Hsp90.

Because only human TR and RAR have been investigated for binding to Hsp90, it is not known whether Hsp90 forms either transient or stable complexes with either TR or RAR in organisms that evolved earlier in the deuterostome line. The above hypothesis suggests examining TR and RAR in frogs, lamprey, Ciona, and sea urchin for forming either transient or stable complexes with Hsp90. Such studies would be relevant to the role of Hsp90 in the evolution of TR and RAR as signals for metamorphosis in deuterostomes.

A role for extreme cooling in vertebrate evolution

The glaciations that ended ~630 and 580 million years ago are either close to or overlap at the time when invertebrates and chordates have been proposed to have diverged from their common ancestor (Ayala and others 1998; Benton and Ayala 2003; Blair and Hedges 2004; Peterson and Butterfield 2005). Relevant to vertebrate evolution are genome-size duplications that have been proposed to occur in the chordate line between cephalochordates (for example amphioxus) and cyclostomes (for example lamprey) (Holland and others 1994; Holland 1999; Dehal and Boore 2005). A duplicated gene and its *cis* regulatory sites can accumulate mutations, without loss of physiological viability to the host. Moreover, duplications at the genome level are especially useful in evolution because they amplify a suite of regulatory proteins that already are coordinated in their actions. If deuterostomes arose before either 630 or 580 million years ago, then one or both of these glaciations may have overlapped a genome-size duplication.

The effect of these intervals of extreme cooling on Hsp90 would allow the expression of pre-existing mutants of duplicated genes of signal-transduction proteins in chordates. For example, adrenal and sex steroid receptors, which have an important role in differentiation and development in vertebrates, evolved by serial gene duplications in a chordate (Baker 1997, 2003, 2004; Laudet 1997; Escriva and others 2000; Thornton 2001). Expression of pre-existing mutants during extreme cooling in the late Proterozoic would add developmental pathways for physiological responses that evolved during the transition of protochordates to primitive vertebrates.

Other environmental influences on signal-transduction proteins

Other interactions in the environment can influence signal-transduction pathways involved in metamorphosis in some metazoans. For example, the ansamycin class of antibiotics, such as geldanamycin and radicicol, inhibit protein chaperoning by Hsp90. These chemicals have been used to study functioning of Hsp90 (Rutherford and Lindquist 1998; Smith and others 1998; Picard 2002; Queitsch and others 2002; Pratt and Toft 2003). These antibiotics and other natural chemicals in the environment could inactivate Hsp90 and release cryptic mutants.

Our hypothesis suggests experiments to study the role of Hsp90 in metamophosis. *Caenorhabditis elegans* offers a good model system to seek changes at a molecular level that would be relevant to disruption of Hsp90 because the fates of individual cell types have been characterized. At this time, the most convenient approach (exposure of embryos to geldanamycin) is not feasible because *C. elegans* Hsp90 does not bind geldanamycin (David and others 2003; Devaney and others 2005), unlike Hsp90 orthologs in yeast, arthropods, and vertebrates. RNA interference can be used to reduce Hsp90 in *C. elegans* (Piano and others 2000). Temperature-sensitive Hsp90 mutants also can be used (Birnby and others 2000; David and others 2003). However, disruption of chaperoning by Hsp90 of mutant signal-transduction proteins in wild-type *C. elegans* embryos also can be accomplished by exposure to elevated temperatures. In addition to visual observation of changes in morphology and timing of development after exposure to elevated temperatures, microarrays can be used to study gene expression in *C. elegans* with mutant signal-transduction proteins. This would permit a study of the effect of disruption of Hsp90 on the development of the nervous and reproductive systems, as well as on longevity in *C. elegans*.

Conflict of interest: None declared.

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