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The Genetic Response to Snowball Earth: Role of HSP90 in the Cambrian Explosion

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Abstract

The events that shaped the Cambrian explosion from 545 to 530 myr ago, when multicellular animals suddenly appeared in the fossil record, are not fully understood. It is likely that the evolution of new transcription factors and other signal transduction proteins that regulated developmental networks were important in the emergence of diverse animal phyla seen in the Cambrian. I propose that one or both extensive glaciations that ended about 670 myr and 635 myr ago were important in the evolution of signal transduction proteins in small animals in the Neoproterozoic/Proterozoic. These glaciations have been called Snowball Earth. One consequence of these glaciations is that they increased the expression of genetic diversity in animals due to the effect of extreme climatic stress on heat shock protein 90 (HSP90). Climatic stress diverted HSP90 from chaperoning the folding and proper intracellular localization of many signal transduction proteins that regulate development in animals. As a result, pre-existing mutant signal transduction proteins and developmental pathways were expressed in animals. Selectively advantageous mutations were fixed in stem group animals and later were a source for the expansion of animal phyla during the Cambrian.

Introduction.

At the beginning of the Cambrian, about 545 myr ago, there is a period of about 15 million years in which the fossil record displays an extraordinary expansion of diverse animal phyla. The brevity of the Cambrian, which is a “blink of an eye” on a geological clock, has been a puzzle to Darwin and subsequent evolutionary biologists (*Fortey et al.*, 1997; *Valentine et al.*, 1999; Conway Morris, 2000a,b; Budd & Jensen, 2000; Budd, 2003; *Grey et al.*, 2003; *Peterson et al.*, 2005). Indeed, the causes of this period in animal evolution still are not fully understood. However, it is thought that geochemical and climatic events were important factors in the emergence of animals in the Cambrian. For example, the Cambrian coincides with an increase in atmospheric oxygen to levels that supported higher metabolism in multicellular animals (Canfield & Teske, 1996; Knoll, 1999; Knoll & Carroll, 1999).

Here I discuss the role in the Cambrian explosion of two extreme glaciations that ended about 670 myr ago and 635 myr ago, during the Neoproterozoic/Proterozoic (*Hoffman et al.*, 1998; *Hyde et al.*, 2000; Runnegar, 2000; Lubick, 2002; *Bodiselitsch et al*., 2005). These extensive glaciations either froze the entire ocean –Snowball Earth (*Hoffman et al.*, 1998) - or most of the ocean - Slushball Earth (*Hyde et al*., 2000; Runnegar, 2000; Lubick, 2002). Each

freezing event was followed by a warm interval that melted the ice. It is likely that this severe cooling created population bottlenecks in Neoproterozoic/Proterozoic animals, leading to the emergence of new organisms during the radiation that repopulated the biosphere, after the glaciers melted (Hoffman et al., 1998; Peterson et al., 2004; 2005).

I propose a genetic consequence of these glaciations, which influenced later evolution of new phyla during the Cambrian: These glaciations stimulated the evolution of new organisms by increasing the expression of novel developmental networks in animals, through their effects on HSP90, a highly conserved, multifunctional protein that is essential for the viability of eukaryotes from yeast to humans (Feder & Hofmann, 1999; Rutherford, 2003; *Sangster et al.,* 2004). This provided a genetic foundation for the explosive increase in the appearance of animal phyla during the Cambrian.

Hypothesis.

Extreme heat and cold alters the conformation of proteins. In the non-native conformation, the binding of deformed proteins to HSP90 disrupts its typical cellular chaperone activity, which, as shown for *Drosophila*, leads to expression of cryptic mutations in genes that regulate differentiation and development (Rutherford & Lindquist, 1998; Cossins 1998; McLaren, 1999; Rutherford, 2003; *Sangster et al.,* 2004). Activation of this mechanism during the extreme cooling and subsequent warming in the late Proterozoic contributed to the evolution of novel developmental networks that led to the diverse multicellular animals that appeared in the Cambrian.

Snowball Earth coincides with evolution of genes that regulate developmental complexity.

Due to uncertainties in molecular clocks for proteins, there is controversy about when the various phyla emerged (Aris-Brosou & Yang, 2003; *Peterson et al.,* 2004; 2005; Blair & Hedges, 2005, *Welch et al.*, 2005). Some propose that arthropods and deuterostomes diverged about 1000 myr ago (Blair & Hedges, 2005); others predict a more recent divergence from 700 to 570 myr ago (*Ayala et al.*, 1998; *Bromham et al.,* 1998; Aris-Brosou & Yang, 2003, *Douzery et al.*;2004; *Peterson et al.,* 2004, Peterson & Butterfield, 2005). Also, Budd and Jensen (2000) propose an orderly development of signaling networks in stem groups during the late Proterozoic, which manifests in the crown groups seen in the Cambrian. In these models, the evolution of various signaling networks in multicellular animals either overlaps or is close to one or more of the two extreme glaciations.

It should be noted that the extent of the glaciations is controversial. There is evidence for diverse life in the ocean near the equator during the Neoproterozoic glaciations (Corsetti et al., 2003; Olicott et al., 2005), which would support a Slushball Earth model. Even in this less extreme model, glaciation would remove or restrict life over much of the Earth. As the glaciers melted, the increased temperature would facilitate the expression of cryptic mutations due to

disruption of the HSP90 from chaperoning the folding of mutant signal transduction proteins. Some of these mutant organisms would flourish during the radiation of life into the rest of the Earth.

HSP90 stabilizes signal transduction proteins in eukaryotes.

Important for the hypothesis is that HSP90 promotes the proper folding and intracellular location of a variety of signal transduction proteins that are inherently unstable (Feder & Hofmann 1999; Rutherford 2003; *Sangster et al.,* 2004). Some of these proteins, such as steroid receptors and aryl hydrocarbon receptors, are ligand-dependent transcription factors; others, such as MyoD and mutated p53, are ligand-independent transcription factors. HSP90 also stabilizes tyrosine kinases, such as src family kinases, and serine/threonine kinases that act in the mitogen-actived protein (MAP) pathway (Pratt & Toft, 1997; *Smith et al.,* 1998; Mayer & Bukau, 1999; Feder & Hofmann, 1999; *Young et al*., 2001; Rutherford, 2003; *Sangster et al.,* 2004). Thus many signal transduction networks that are important in differentiation and development in animals depend on HSP90 for functional integrity.

HSP90 promotes the proper folding and intracellular localization of mutant signal transduction proteins, which would be destabilized by sequence mutations. In this way, HSP90 maintains the normal phenotype in the presence of underlying genetic variation, a process called canalization (Waddington, 1942; Wilkins, 1997).

Under stress, HSP90 is diverted from buffering mutations in signal transduction proteins and towards 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.

Cold and heat compromise HSP90-repression of cryptic mutations in signal transduction proteins.

A connection of HSP90 and extreme cooling in the late Proterozoic to the Cambrian explosion comes from a series of experiments by Rutherford and Lindquist with *Drosophila* that were heterozygous for mutant HSP90 (Rutherford & Lindquist, 1998; Cossins, 1998; McLaren, 1999). 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^{o}C or 30^{o}C instead of the normal 25^{o}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.

This provides a mechanism for intervals of extreme cooling and subsequent warming to increase developmental diversity in stem group animals (Budd & Jensen, 2000) prior to the beginning of the Cambrian.

Testing the hypothesis.

Discovery of metazoan fossils in strata that are close to one or both of the intervals of Snowball Earth will support the hypotheses. Despite the difficulty in finding fossils of microscopic animals (Butterfied, 2003), fossils for bilaterians from 580 to 600 myr ago in the Doushantuo Formation have been reported (*Chen et al.;* 2004a). Although there is some controversy about the nature of these fossils (Bengston & Budd, 2004; *Chen et al.*, 2004b), this recent finding is encouraging for further studies to uncover more evidence about metazoans that lived near the end of Snowball Earth.

Biological tests of this hypothesis can use protocols developed by Rutherford and Lindquist (1998) in demonstrating that the release of HSP90 from chaperoning signal transduction proteins was a mechanism for morphological evolution in *Drosophila*. However, as noted later, the *Drosophila* mutants were "monstrous" (*Queitsch et al.*, 2002). Indeed, none of the mutant flies appeared to have a biological advantage over wild-type flies. Thus, it has been questioned whether compromising HSP90 stabilization of mutant signal transduction proteins has an evolutionary effect in the real world (Mitchell-Olds & Knight 2002).

However, it is not surprising that *Drosophila*, an animal with complex regulatory networks and morphology would yield monster mutants. Complexity restrains future evolutionary novelty (Jacob, 1977; Duboule & Wilkins 1998; Budd & Jensen, 2000). Pre-Cambrian animals were simpler. In some of these animals, new networks could evolve without creating monsters. Even the simplest modern animals are not likely to be the same genetically as their pre-Cambrian ancestors, which makes it difficult to replicate conditions during Snowball Earth. With that caveat, *Caenorhabditis elegans* offers a good model system to seek changes at a molecular level that would be relevant to the evolution of complexity in multicellular animals because the fates of individual cell types have been characterized.

HSP90 could be disrupted by exposure of *C. elegans* embryos to geldanamycin, which has been successfully used to reveal cryptic mutations in flies (Rutherford & Lindquist, 1998) and plants (*Queitsch et al.*, 2002). In addition to visual observation of changes in morphology and timing of development in the presence of geldanamycin, microarrays are available to study gene expression in *C. elegans*. This would permit a study of the effect of disruption of HSP90 on development of the nervous and reproductive systems, as well as longevity in *C. elegans* (Kenyon, 2005).

The discussion in this paper also is relevant to the effect of extreme climate on the evolution of new developmental pathways that were important in transitions to more complex organisms earlier in the Proterozoic (Szathmary & Maynard Smith, 1995).

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