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RESEARCH PERSPECTIVE

Developmental arrest: from sea urchins to seeds

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Abstract

The phenomenon of dormancy extends beyond the boundaries of the plant kingdom. While plant biologists typically associate dormancy-breaking treatments only with seeds, buds or tubers, these chemicals and environmental stimuli have much broader activity as general terminators of developmental arrest in other, non-plant species. The activation of growth by these treatments is associated with signal transduction processes, metabolic upregulation and changes in gene expression, in addition to other events that may or may not be species specific. The study of both the classic and current developmental arrest literature beyond the boundaries of plant biology may be helpful in generating useful ideas and analogies for meaningful experimental progress towards understanding seed dormancy.

Keywords: developmental arrest, diapause, dormancy-breaking chemicals, egg activation, seed dormancy, spore dormancy

Introduction

To survive adverse environmental conditions (e.g. cold, water limitations, oxygen deprivation, etc.) many species enter an arrested state (e.g. Henis, 1987; Cáceres, 1997 and references therein). Generally, this state can be primarily metabolic arrest alone (quiescence) (Hochachka and Guppy, 1987; Guppy and Withers, 1999) or in combination with developmental arrest (dormancy or diapause)(e.g. Hand, 1991), where an individual is genetically programmed and specifically prevented from entering subsequent growth stages of its life cycle. Organisms entering developmental arrest generally do so before attaining their adult form. For example, developmental arrest is observed in the egg (*Arbacia* sp.) (Epel, 1989), blastocyst (*Capreolus capreolus*) (Renfree, 1978), cyst (*Artemia* sp.) (Drinkwater and Crowe, 1987), larval (*Haemonchus contortus*) (Petronijevic *et al*., 1986), gemmule (*Eunapius fragilis*) (Loomis *et al*., 1996), pupal (*Sarcophaga crassipalpis*) (Denlinger *et al*., 1980), seed (Bewley and Black, 1982, 1994) and spore (*Phycomyces blakesleeanus*) (Thevelein *et al*., 1979) stages. However, a developmentally arrested state is not an obligatory component of all life cycles (Sussex, 1978).

Developmental arrest occurs when an organism enters a form resistant to adverse conditions. As growth and development cease, metabolic activities are concertedly down-regulated to minimal levels to retain viability. An ametabolic state does not generally occur, as metabolism can be detected in both dry and hydrated systems (Bewley and Black, 1982; Hand and Gnaiger, 1988; Footitt *et al*., 1995; Hand and Hardewig, 1996; Brooks and Storey, 1997). Development does not continue when optimum environmental conditions simply resume. An activating stimulus is required to terminate developmental arrest (i.e. to break dormancy). This activating stimulus is usually not required for additional further growth. The nature of developmental arrest has been reviewed in a number of model systems, such as eggs (Loeb, 1913; Epel, 1989, 1990), insects (Jungreis, 1978), seeds (Bewley and Black, 1982; Simpson, 1990; Hilhorst, 1995, 1998; Bewley, 1997), spores (Sussman and Halvorson, 1966) and diapausing aquatic animals (Hand and Podrabsky, 2000).

Therefore, dormancy is a phenomenon common

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from the monera to the animal kingdom. For almost 100 years dormancy research has tended to remain within the bounds of its respective kingdom. This has held back progress in a field that directly and indirectly affects human health and welfare. Progress in our laboratory was contained within the same boundaries until we discovered the sea urchin egg activation research and some early 20th-century seed papers buried in the literature. The conceptual flood gates then opened, and by reviewing the field of dormancy-breaking chemicals without regard to kingdom or species, a pattern emerged that may help to cast light on the underlying mechanism of physiological dormancy-breaking. In the past few years such ideas have started to spread, primarily from the field of egg activation, bringing an increasing awareness of advances in diverse and seemingly unrelated model systems. Focusing upon genetically programmed developmental arrest, this review highlights the widespread nature and commonalities of chemical-based dormancy-breaking treatments and a few consequences of such treatments.

Early development of chemical-based dormancy-breaking treatments

Towards the end of the 19th century, the properties of chemicals capable of perturbing biological systems attracted increasing attention. Fungal spore susceptibility to toxic acids was related to the presence of the undissociated acid form (Clark, 1898). Cell permeability to weak bases was equated with their degree of dissociation and lipophilicity (Harvey, 1911). Alcohol partition coefficients (lipophilicity) and anaesthetic potency were found to increase with carbon number (Overton, 1901). At the same time, developmental biologists recognized these properties as affecting the activity of chemicals that terminated developmental arrest (Loeb, 1913).

Common thematic features of chemical-based dormancy-breaking treatments

Structural features

A large number of dormancy-breaking chemicals are active in surprisingly diverse model systems (examples in Table 1). Small molecular weight substances, known to many of us only as seed dormancy-breaking chemicals (e.g. weak acids, aldehydes, ketones and alcohols), terminate developmental arrest in a variety of species. From this survey, the common structural features of dormancy-breaking chemicals can be identified (see following sections). In our laboratory, consideration of these features increased both the successful use and discovery of seed dormancy-breaking chemicals. Lipophilicity plots proved especially useful for predicting the concentration range for dormancy-breaking activity of previously untested chemicals (Cohn, 1989, 1997; Cohn *et al*., 1989, 1991). In addition to lipophilicity, the nature and position of oxidizable functional groups were critical factors governing activity (reviewed in Cohn, 1996a).

The pH effect and the impact of dissociation constants

Acid scarification, a common seed treatment, terminates developmental arrest in systems other than seeds. A number of reports have shown that inorganic acids at pH 2 break dormancy (Loeb, 1913; Lillie, 1926; Sibilia, 1930; Bingham and Meyer, 1979; Adkins *et al*., 1985; Petronijevic *et al*., 1986). Whether activation is a result of acid scarification/mechanical injury or acid loading is not clear. Weak acids and bases also break dormancy under milder, more controlled conditions in a pH-dependent manner, and this effect is related to their dissociation constants (p*K*s). Dormancy-breaking activity requires the presence of the uncharged chemical species. This has been recognized in a number of model systems (Loeb, 1913; Lillie, 1926; Toole and Cathey, 1961; Palevitch and Thomas, 1976; Cohn and Hughes, 1986; Petronijevic *et al*., 1986; Van Mulders *et al*., 1986; Cohn *et al*., 1987; Petronijevic and Rogers, 1987a).

Contact time

The application of dormancy-breaking chemicals as a pulse is more effective than continuous contact (Loeb, 1913; Lillie, 1926; Zagorski and Lewak, 1984; Cohn and Hughes, 1986). This is almost certainly due to the detrimental effect of prolonged exposure to dormancy-breaking chemicals on normal development (Loeb, 1913; Lillie, 1926; Mayer and Evenari, 1953). These reports also confirm what has been commonly known for many seeds: conditions for the termination of developmental arrest can be very different from those supporting growth.

Lipophilicity and molecular size

In several model systems the dormancy-breaking activity of organic acids and their derivatives is correlated with their lipophilicity (Loeb, 1913; Thevelein *et al*., 1979; Belmans *et al*., 1983; Taylorson, 1988; Cohn *et al*., 1989). In seeds, this correlation is modified by a functional group effect, with weak acids being more active (Cohn *et al*., 1989). The activity of some compounds (e.g. inorganic nitrogen

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Table 1. Chemicals showing dormancy-breaking activity and the kingdoms in which they are found to be active. In each kingdom, the number designates a species where the appropriate chemical shows activity. The chemicals and species identified are representative only; other examples can be found in the cited literature. Species are identified in the species key below. Within each kingdom, species are arranged by phyla

	Kingdom				
Chemical	Monera	Protista	Fungi	Plantae	Animalia
Alkanes					
Butane	2				
Pentane	2				10
Hexane	2				9, 10
Heptane					10
Iso-octane					10
Cyclohexane					10
Alkenes					
Ethylene				10, 11, 13, 14	
Propylene	2			13, 14	
Propadiene				14	
1-Butene	2				
1-Hexene					10
Monocarboxylic acids					
Methanoic acid	2		1	7	4, 12, 15
Ethanoic acid	2	2, 3	1,3	1, 3, 7	6, 12, 13, 15
Propanoic acid	2		1, 4	7	12, 15
2-Propenoic acid			1		
Butanoic acid	2		1, 3, 4	1, 2, 7	12, 13, 15
Isobutanoic acid			1, 3	7	12
Isopentanoic acid			1	7	12
Pentanoic acid	2		1, 3	7	12, 15
Hexanoic acid	2		1	7	12, 15
Isohexanoic acid					12
Heptanoic acid					15
Octanoic acid					15
Nonanoic acid					15
Decanoic acid					15
Palmitic acid			9		
Oleic acid			8, 9		
Linoleic acid			8, 9		10
Linolenic acid			8		
Dicarboxylic acids					
Fumaric acid		7			
Malonic		7			
Oxalic acid		7			6, 15
Succinic acid		7		7	
Tricarboxylic acids					
Citric acid		7			15
Hydroxyacids					
Glycolic acid				7	
Ascorbic acid				3, 4	
Lactic acid				7	12
β-Hydroxybutyric acid				7	15
Aldehydes					
Formaldehyde				6	
Acetaldehyde				3, 7	
Propionaldehyde				7	
Pentanal			9		
Hexanal			8, 9	15	
2,4-Hexadienal			8		
Heptanal			7, 9		
Octanal			7, 8, 9	15	
Nonanal			7, 9		

Continued over

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Table 1. *Continued*

	Kingdom				
Chemical	Monera	Protista	Fungi	Plantae	Animalia
Decanal			9		
Hexadecanal			8		
Esters					
Methyl formate				7	
Methyl propionate				7	
Ethyl acetate				7	10
Ethyl butyrate			2		
Ketones					
Propanone			2	7[a], 9	9, 10
Butanone					10
2-Pentanone					10
3-Pentanone					10
1-Penten-3-one			8, 9		
2-Hexanone			6, 7		
2-Heptanone			6, 7	15	
2-Octanone			5, 6, 7	15, 16	
2-Nonanone			5, 6, 7	15, 16	
Alcohols					
Methanol	3		1, 2	7, 9, 10, 11	
Ethanol			1, 2	3, 4, 5, 7, 8, 11	8, 12
Propanol			1, 2	3, 4, 5, 7, 8	12
Isopropanol			2	7, 11, 16	
2-Propen-1-ol				4	
Butanol	2		1, 2	3, 4, 5, 7	10, 12
Isobutanol				7[a]	
Pentanol	2		1, 2	5, 7, 8	
Hexanol	2		1, 2	7[a]	
Heptanol			1, 2	7[a]	
Octanol			1, 2	7[a]	
3-Octanol					1
1-Octene-3-ol					1
Nonanol			7		
2-Nonanol			7	15, 16	
3-Nonanol				16	
1-Nonen-3-ol				16	
Amines					
Hydroxylamine	2			3, 7, 10	
Methylamine	1				5
Ethylamine	2				5
Propylamine				7[a]	
Butylamine					5, 13
Heptylamine	2				
Octylamine	2				
Decylamine	2				
Dodecylamine	2				
Ethers					
Diethyl ether				9, 11	8, 10, 11
Aromatics					
Benzene					10
Benzoic acid				7	12, 15
Benzyl acetate			5, 7		
Benzaldehyde			5, 7	16	
Benzyl alcohol				5, 8	
Benzyl amine					5, 13
Phenol			1		
Salicylic acid			1	7	12
Salicylhydroxamic acid				13, 15	
Methyl salicylic acid			5, 7	10	

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Table 1. *Continued*

Chemical	Kingdom				
	Monera	Protista	Fungi	Plantae	Animalia
Salicylaldehyde			7		
Toluene					10
Xylene					10
Xylol					8, 11
Inorganics					
Ammonia	1		1	3, 7[a], 12	2, 3, 4, 5, 13
Azide				3, 7, 10	
Carbon dioxide		1, 2, 3, 4, 5, 6		5, 7, 9	2, 3, 4, 7, 12, 13, 14, 15
Cyanide				7, 10, 13	12, 14
Hydrogen peroxide				6	7
Hydrogen sulphide		5		7[a]	4
Nitrate	2	2, 3		3, 10	12
Nitrite				3, 7, 10	
Nitric oxide		5			
Nitrogen dioxide				7	

[a] Unpublished data.

Key to species:

MONERA

Schizophyta: 1, *Bacillus cereus* endospore (Preston and Douthit, 1988); 2, *Bacillus megaterium* endospore (Levinson and Sevag, 1953; Rode and Foster, 1961, 1965); 3, *Thermoactinomyces vulgaris* endospore (Kirillova *et al*., 1974).

PROTISTA

Sarcomastigophora: 1, *Giardia muris* cyst (Schaefer *et al*., 1984); 2, *Hartmannella rhysodes* cyst (Datta, 1979); 3, *Schizopyrenus russelli* cyst (Datta, 1979).

Apicomplexa: 4, *Eimeria bovis* cyst (Jensen *et al*., 1976); 5, *Eimeria stiedai* cyst (Jensen *et al*., 1976); 6, *Eimeria tenella* cyst (Nyberg *et al*., 1968; Jensen *et al*., 1976).

Ciliophora: 7, *Pleurotricha lanceolata* cyst (Jeffries, 1956, 1962).

FUNGI

Amastigomycota: 1, *Phycomyces blakesleeanus* conidiospore (Thevelein *et al*., 1979, 1983; Van Mulders *et al*., 1986); 2, *Neurospora tetrasperma* ascospore (Belmans *et al*., 1983); 3, *Hygrophorus russula* basidiospore (Ohta, 1988); 4, *Tricholoma flavovirens* uredospore (French, 1984); 6, *Uromyces vignae* uredospore (French, 1984); 7, *Uromyces rumicis* uredospore (French *et al*., 1986); 8, *Alternaria alternata* conidiospore (Harman *et al*., 1980); 9, *Fusarium solani* conidiospore (Harman *et al*., 1980).

PLANTAE

Phaeophycophyta: 1, *Fucus vesiculosus* egg (Overton, 1913); 2, *Sargassum piluliferum* egg (Hiroe and Inoh, 1954).

Anthophyta: 3, *Avena fatua* seed (Adkins *et al*., 1984a, b, 1985; Cairns and De Villiers, 1986); 4, *Avena sativa* seed (Corbineau *et al*., 1991); 5, *Echinochloa crus-galli* seed (Taylorson, 1988; Leather *et al*., 1992); 6, *Hordeum vulgare* seed (Hareland and Madson, 1989; Fontaine *et al*., 1994); 7, *Oryza sativa* seed (Tseng, 1964; Major and Roberts, 1968; Cohn *et al*., 1983, 1987, 1989; Cohn and Castle, 1984; Cohn and Hughes, 1986); 8, *Panicum capillare* seed (Taylorson, 1989); 9, *Panicum dichotomiflorum* seed (Taylorson and Hendricks, 1979; Taylorson, 1980); 10, *Amaranthus albus* seed (Hendricks and Taylorson, 1974; Taylorson, 1979); 11, *Amaranthus retroflexus* seed (Taylorson, 1979, 1989; Schonbeck and Egley, 1980); 12 *Berbera verna* seed (Hendricks and Taylorson, 1974); 13, *Lactuca sativa* seed (Brooks *et al*., 1985; Abeles, 1986); 14, *Portulaca oleracea* seed (Taylorson, 1979); 15, *Rumex acetosella* seed (French and Leather, 1979); 16. *Rumex crispus* seed (French and Leather, 1979; Taylorson, 1984; French *et al*., 1986).

ANIMALIA

Nematoda: 1, *Bursaphelenchus xylophilus* juvenile (Matsumori *et al*., 1989); 2, *Ascaris suum* juvenile (Petronijevic and Rogers, 1987a); 3, *Haemonchus contortus* juvenile (Petronijevic *et al*., 1986; Petronijevic and Rogers, 1987a, b); 4, *Nematospiroides dubius* juvenile (Petronijevic *et al*., 1986).

Annelida: 5. *Polynoe* sp. egg (Loeb, 1913); 6, *Thalassema mellita* egg (Loeb, 1913).

Arthropoda: 7, *Artemia franciscana* cyst (Drinkwater and Crowe, 1987; Clegg *et al*., 1996); 8, *Melanoplus differentialis* egg (Slifer, 1946); 9, *Manduca sexta* pupae (Denlinger *et al*., 1980); 10, *Sarcophaga crassipalpis* pupae (Denlinger *et al*., 1980); 11, *Loxostege sticticalis* pupae (Pepper, 1937).

Echinodermata: 12, *Asterias forbesii* egg (Lillie, 1910, 1913, 1926, 1927); 13, *Arbacia* sp. egg (Lyon, 1903; Loeb, 1913; Harding, 1951); 14, *Paracentrotus lividus* egg (Lyon, 1903; Loeb, 1913); 15, *Strongylocentrotus purpuratus* egg (Loeb, 1913).

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compounds) appears to be better related to molecular size rather than lipophilicity (Cohn *et al*., 1989). Overall, it is somewhat disappointing that features, identified by Loeb (1913) in the activation of sea urchin eggs (contact time, dissociation constant and lipophilicity), have taken so long to be recognized in other model systems, especially seeds. On the other hand, detailed structure–activity studies of chemicals that activate eggs have yet to be conducted.

Is there a common mechanistic basis for cross-kingdom chemical activities?

Many plant species exhibit physiological seed dormancy, i.e. resulting not from an impermeable seed coat, physical restraint or an immature embryo, but from the consequences of a developmental programme. Physiological dormancy can be broken in response to environmental, physical and chemical agents (Bewley and Black, 1994). Several hypotheses have been presented to explain the dormancy-breaking ability of these agents (reviewed in Bewley and Black, 1982, 1994; Cohn, 1987). In the case of chemical agents, hypotheses tend to centre on specific components of metabolism (e.g. Roberts and Smith, 1977; Taylorson and Hendricks, 1980/81; Esashi *et al*., 1981a, b). These hypotheses attempt to explain the dormancy-breaking action of specific groups of compounds. However, until recently they have not been able to address the fundamental question of why a wide range of apparently unrelated chemicals are able to break dormancy (see Cohn and Hilhorst, 2000).

Of the known dormant forms in the kingdoms of life, the majority are activated by representatives from many of the chemical classes shown in Table 1. A notable exception has been the alkanes, which appear to have no dormancy-breaking activity in seeds (Taylorson, 1979; Abeles, 1986; Cohn *et al*., 1989). Initially, it may appear unusual that such a wide variety of chemicals should be active in such a diverse number of species. However, this can be accommodated if similar dormancy-breaking mechanisms operate in all kingdoms.

Markers for dormancy-breaking and early germination events

In previous publications, we have documented the need for physiological/biochemical markers as guideposts for the time course that seeds experience as they progress from dormancy to germination processes as a consequence of the application of dormancy-breaking chemicals (Footitt and Cohn, 1992, 1995; Cohn and Footitt, 1993; Footitt *et al*., 1995; Cohn, 1996a, b). These have included changes in tissue pH, levels of fructose 2,6-bisphosphate, as well as uptake kinetics and metabolism of dormancy-breaking chemicals. Review of the historical literature indicates that some of these putative markers have wider relevance beyond the world of seeds.

Intracellular pH

Many developmentally arrested systems exhibit a change in internal pH upon activation (Table 2). In unicellular systems such as sea urchin and *Xenopus* eggs, one of the early events of fertilization or artificial activation is an increase in intracellular pH (Grainger *et al*., 1979; Whitaker and Steinhardt, 1982; Busa and Nuccitelli, 1984; Charbonneau and Grandin, 1989; Epel, 1989; Freeman and Ridgway, 1993; Miller and Epel, 1999). By contrast, intracellular pH decreases upon activation of dormant multicellular systems, such as diapause cysts of the brine shrimp, *Artemia franciscana* (Drinkwater and Crowe, 1987), larvae and juveniles of the nematodes, *Caenorhabditis elegans* and *Haemonchus contortus* (Petronijevic and Rogers, 1987b; Wadsworth and Riddle, 1988), and upon proliferation of Syrian hamster embryo cells (Isfort *et al*., 1993, 1995). In plants, intracellular pH is also higher in dormant than in non-dormant tissues, e.g. Jerusalem artichoke tuber buds (Gendraud and Lafleuriel, 1983), red rice embryos (Footitt and Cohn, 1992) and barley aleurone cells (Van Beckum *et al*., 1993). The pH of dormant seed embryos decreased during dormancy-breaking treatments and germination (Table 2). These observations identify changes in intracellular pH as a potential marker for the change in developmental pattern.

Small molecular weight substances

At some time interval after the application of a dormancy-breaking chemical to seeds, there must be a resumption of metabolic activity associated with normal germination that occurs prior to radicle emergence. One of the challenges of seed dormancy research is to avoid confusing activities associated with the dormancy-breaking process in contrast to these germination events. Identification of biochemical markers to delineate these processes would be of significant interpretive value. Because of the numerous synthetic processes required for germination, particularly transcription and translation, one of the earliest manifestations of the germination process would be increased carbon flux through energy-generating pathways such as glycolysis (Botha *et al*., 1992) and an increase in the metabolic regulator, fructose 2,6-bisphosphate (Fru 2,6-P_2), which activates pyrophosphate:fructose 6-phosphate 1-phosphotransferase (PP_i-PFK) and

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Table 2. Examples of species exhibiting a change in pH upon termination of developmental arrest

Species	pH	Reference
Unicellular systems		
Strongylocentrotus purpuratus	⇑	Johnson *et al*. (1976)
Xenopus laevis	⇑	Nuccitelli *et al*. (1981)
Carcinus maenas	⇑	Hervé *et al*. (1989)
Urechis caupo	⇑	Gould and Stephano (1993)
Multicellular systems		
Crataegus gloriosa	⇓	Eckerson (1913)
Avena fatua	⇓	Atwood (1914)
Tilia americana	⇓	Rose (1919)
Acer saccharinum	⇓	Jones (1920)
Juniperus virginiana	⇓	Pack (1921)
Helianthus tuberosa	⇓	Gendraud and Lafleuriel (1983)
Artemia franciscana	⇓	Drinkwater and Crowe (1987)
Haemonchus contortus	⇓	Petronijevic and Rogers (1987b)
Oryza sativa	⇓	Footitt and Cohn (1992)

inhibits cytosolic fructose 1,6-bisphosphatase (Van Schaftingen, 1987; Stitt, 1990). PP_i-PFK-specific activity can increase when oxygen is limiting during seedling growth (Mertens *et al*., 1990; Mertens, 1991), and this could also occur in the dense tissues of a germinating seed, as seems to be the case in *Phaseolus vulgaris* (Botha and Small, 1987). In dormant tubers and roots, restoration of metabolic activity led to an increase in Fru 2,6-P_2 (Van Schaftingen and Hers, 1983; Kowalczyk, 1989). In *Phycomyces blakesleeanus* spores and seeds of *Avena sativa*, dormancy-breaking chemical treatments induced a transient rise in Fru 2,6-P_2 (Van Laere *et al*., 1983; Larondelle *et al*., 1987). In non-dormant *Avena sativa*, a transient increase was also seen in the germination phase prior to radicle protrusion (Larondelle *et al*., 1987). The *Avena sativa* study used intact caryopses. Thus, the transient increase may result from anaerobic conditions in the embryo resulting from oxygen uptake by microflora or the glumes (Lenoir *et al*., 1986; Briggs and McGuinness, 1992), although under similar conditions no transient increase was seen in barley (Thornton, Footitt and Bryce, unpublished data). After the application of nitrite or propionaldehyde to break dormancy in red rice grains, a dramatic increase in [Fru 2,6-P_2] was observed within 4 h of chemical treatment. However, other dormancy-breaking chemicals did not elicit increased [Fru 2,6-P_2] until the time of radicle protrusion, possibly due to inhibitory side-effects of dormancy-breaking weak acids and their esters on glycolytic activity (Footitt and Cohn, 1995). Considering all of this information together, Fru 2,6-P_2 may have potential value as a marker for increased metabolic activity during dormancy-breaking and germination in some well-characterized situations. However, the differential sensitivity of seed tissue [Fru 2,6-P_2] to various chemical and environmental factors prevents adoption of [Fru 2,6-P_2] as a 'fail-safe', universal marker for dormancy-breaking or the dormant/non-dormant transition.

In dry wild oat (*Avena fatua*) seeds, the adenosine triphosphate (ATP) concentration is lower in dormant than in non-dormant seeds (Adkins and Ross, 1983). On hydration, differences in developmental state were not reflected in the ATP levels or energy charge (Adkins and Ross, 1983; Larondelle *et al*., 1987; Côme *et al*., 1988). Gibberellic acid (Adkins and Ross, 1983) and ethanol (Larondelle *et al*., 1987) broke dormancy, but ATP levels were the same as in dormant seeds. However, in dormant *Kalanchoë blossfeldiana* seeds, a non-saturating dose of red light increased ATP content without breaking dormancy, in effect inducing a physiological response in a dormant seed (De Greef *et al*., 1989; Dedonder *et al*., 1992). As ATP levels are highly buffered, the level of unbound adenosine diphosphate (ADP) has been suggested as a more useful measure of energy status (Guppy *et al*., 1994). Results to date suggest that ATP content and energy charge of seeds may not be useful as uniform markers for the transition between the dormant and germinating states.

Ammonia production is a recently identified marker for the germination phase that merits further investigation. In non-dormant *Arabidopsis thaliana* seed, ammonia levels increased rapidly 24 h prior to radicle emergence (Garciarrubio *et al*., 1997; Garciarrubio, personal communication). Further work is required to determine whether such an increase occurs in other species and the timing of such an increase in arrested seeds after a dormancy-breaking treatment.

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Enzyme activity

Enzyme levels and activities might be expected to increase in both the dormant/non-dormant transition and the germination phase. To date, studies on seeds have failed to provide an enzyme as a transitional developmental marker between the dormant and germinating states. This is not the case in other model systems. In tubers, enzyme activities differ between tissue from dormant tubers and tissue slices aged *in vitro* to induce respiration. In aged tissue, the activity of glycolytic enzymes increased, and their distribution changed from the soluble to the particulate fraction (Moorhead and Plaxton, 1988). In animal systems, studies on the activation of sea urchin and clam eggs have also found changes in enzyme activity and location. Total *in vitro* activity of glucose 6-phosphate dehydrogenase (G6PD) was the same in extracts from fertilized and unfertilized eggs. However, upon fertilization, its localization changed from the insoluble (inactive) to soluble (active) fraction (Isono, 1963; Isono *et al*., 1963; Ii and Rebhun, 1982; Swezey and Epel, 1986, 1995). If G6PD was assayed *in situ* using permeabilized eggs, the enzyme activity increased rapidly following fertilization, as did NADPH levels (Epel, 1989; Rees *et al*., 1996). These egg experiments suggest the need for refinement of the 'grind and find' approach previously used in seed studies. (As a side issue, these egg activation data support an important role of the pentose shunt following fertilization in sea urchin. Therefore, there still may be some life in the pentose shunt hypothesis regarding seed dormancy!).

By identifying a series of markers during and following a dormancy-breaking chemical treatment, a time-line of events can be constructed. The position of events on the time-line will suggest other associated events as markers. Hence, the time-line will also fulfil a predictive function. Ultimately, this approach will make it possible to unravel the events that make up the dormancy-breaking process and identify the transition points between the developmental states.

Overview

While there is a tendency to view dormancy-breaking events as a single linear time course, it now seems that multiple, rapidly interacting paths with different activation kinetics are triggered in response to a single dormancy-breaking signal (Footitt and Cohn, 1992, 1995; Footitt *et al*., 1995; Cohn, 1996a) and during the germination process as well (Alvarado *et al*., 2000; Bradford *et al*., 2000). If constitutive metabolism is activated rapidly above the cellular maintenance level after application of a dormancy-breaking chemical, it is intriguing to ponder what regulatory changes are occurring at the level of transcription and translation (including post-translation) to the control of gene expression during the transition from the dormant to non-dormant state and subsequent germination (Morris *et al*., 1991; Goldmark *et al*., 1992; Hance and Bevington, 1992; Dyer, 1993; Johnson *et al*., 1995; Li and Foley, 1995, 1996; Aalen, 1999; Holdsworth *et al*., 1999; Alvarado *et al*., 2000; Bradford *et al*., 2000).

In addition, one must further consider the role of abscisic acid (ABA), produced in the embryo, which is required to induce dormancy (e.g. LePage-Degivry and Garello, 1992; Hilhorst, 1995; McCarty, 1995; Koornneef *et al*., 1998). ABA induces increased cellular pH (Gehring *et al*., 1990; Van der Veen *et al*.,1992) and the expression of dormancy-associated genes (Morris *et al*., 1991; Goldmark *et al*., 1992; Li and Foley, 1995), including the gene for the transcription factor VP1 and its homologues which control embryo maturation and, in some cases, developmental arrest (McCarty, 1995; Jones *et al*., 1997). This suggests the possible involvement of increased embryo pH in the expression of genes responsible for the induction/maintenance of seed dormancy. In this respect, it is intriguing that dormancy-breaking weak acids acidify the internal pH (Cohn *et al*., 1989; Footitt and Cohn, 1992) and inhibit ABA-induced gene expression (Van der Veen *et al*., 1992). In contrast, gibberellic acid-induced gene expression did not appear to be pH sensitive (Van der Veen *et al*., 1992; Heimovaara-Dijkstra *et al*., 1995). These data suggest that internal pH may act as a modulator of some developmental signals, as in the proliferation of Syrian hamster embryo cells, where transient intracellular acidification is indispensable for platelet-derived growth factor-induced proliferation (Isfort *et al*., 1995).

The role of cellular acidification in the loss of dormancy may be to reduce the expression of ABA-related genes, e.g. *VP1* and *PKABA1* homologues. In dormant wild oats, transcription of the *afVP*1 homologue is reduced as a result of dry after-ripening and increased on induction of secondary dormancy (Jones *et al*., 1997, 2000). VP1, as well as PKABA1, represses transcription of germination-specific amylase genes (Hoecker *et al*., 1995; Walker-Simmons, 1998, 2000; Gómez-Cadenas *et al*., 1999). VP1 has also been implicated in repression of key glyoxylate cycle genes (isocitrate lyase and malate synthase) during seed development, thus restricting reserve lipid breakdown prior to germination (Paek *et al*., 1998). Thus, it is possible that VP1, PKABA1 and their homologues have a wider role in repressing the expression of germination-specific genes. The effect of dormancy-breaking chemicals may be to down-regulate expression of *VP1* gene homologues or to interfere with the activity of the transacting factor itself, via cellular acidification. The slow rate of

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germination observed following some dormancy-breaking treatments (Footitt and Cohn, 1992, 1995; Myers *et al*., 1997) may result from the effects of residual VP1 homologue gene products, especially in highly dormant lines (Jones *et al*., 1997), and the secondary toxicological effects of dormancy-breaking chemicals.

To conclude, we have begun to identify potential molecular, biochemical and physiological markers that can serve as orientation points for further dissection of the dormancy-breaking process, the dormant/non-dormant transition, and germination processes prior to radicle emergence in seeds. While most of the markers identified to date are components of the germination process, some progress has been made concerning the progression of seeds from the dormant to the non-dormant state. Based upon parallels to the termination of development arrest in a wide range of organisms in addition to plants, it is highly likely that general components of the current signalling paradigm (calcium, G proteins, nitric oxide, protein kinases and phosphatases, etc.) will play a significant role, as already described extensively for the aleurone model system (Ritchie *et al*., 2000). The results of the literature survey presented here suggest a role for seed cytosolic pH changes. For future seed research, it will be important to confirm experimentally that existing markers are widely relevant to a range of species with various dormancy syndromes, as would be predicted from our cross-kingdom review of activating chemicals. As dormancy appears to be so similar in many embryonic systems, it is also intriguing to wonder whether homologues to genes such as *VP1* and *PKABA1* fulfil related functions in *Artemia* cysts, nematodes, unfertilized oocytes and diapausing insects.

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References

Aalen, R.B. (1999) Peroxiredoxin antioxidants in seed physiology. *Seed Science Research* **9**, 285–295.

Abeles, F.B. (1986) Role of ethylene in *Lactuca sativa* cv 'Grand Rapids' seed germination. *Plant Physiology* **81**, 780–787.

Adkins, S.W. and Ross, J.D. (1983) Adenosine triphosphate and adenylate energy charge in relation to dormancy of wild oat seeds. *Canadian Journal of Botany* **61**, 3349–3354.

Adkins, S.W., Naylor, J.M. and Simpson, G.M. (1984a) The physiological basis of seed dormancy in *Avena fatua*. V. Action of ethanol and other organic compounds. *Physiologia Plantarum* **62**, 18–24.

Adkins, S.W., Simpson, G.M. and Naylor, J.M. (1984b) The physiological basis of seed dormancy in *Avena fatua*. III. Action of nitrogenous compounds. *Physiologia Plantarum* **60**, 227–233.

Adkins, S.W., Simpson, G.M. and Naylor, J.M. (1985) The physiological basis of seed dormancy in *Avena fatua*. VII. Action of organic acids and pH. *Physiologia Plantarum* **65**, 310–316.

Alvarado, V., Nonogaki, H. and Bradford, K.J. (2000) Expression of endo-ß-mannanase and SNF-related protein kinase genes in true potato seeds in relation to dormancy, gibberellin and abscisic acid. pp. 347–364 *in* Viémont, J.-D.; Crabbé, J. (Eds) *Dormancy in plants: From whole plant behaviour to cellular control*. Wallingford, CABI Publishing.

Atwood, W.M. (1914) A physiological study of the germination of *Avena fatua*. *Botanical Gazette* **17**, 386–414.

Belmans, D.L., Van Laere, A.J. and Van Assche, J.A. (1983) Effect of *n*-alcohols and high pressure on the heat activation of *Neurospora tetrasperma* ascospores. *Archives of Microbiology* **134**, 49–51.

Bewley, J.D. (1997) Seed germination and dormancy. *Plant Cell* **9**, 1055–1066.

Bewley, J.D. and Black, M. (1982) *Physiology and biochemistry of seeds in relation to germination. Vol. 2. Viability, dormancy and environmental control*. New York, Springer-Verlag.

Bewley, J.D. and Black, M. (1994) *Seeds. Physiology of development and germination* (2nd edition). New York, Plenum Press.

Bingham, A.K. and Meyer, E.A. (1979) *Giardia* excystation can be induced in vitro in acidic solutions. *Nature* **277**, 301–302.

Botha, F.C. and Small, J.G.C. (1987) Comparison of the activities and some properties of pyrophosphate and ATP dependent fructose-6-phosphate 1-phosphotransferases of *Phaseolus vulgaris* seeds. *Plant Physiology* **83**, 772–777.

Botha, F.C., Potgieter, G.P. and Botha, A.-M. (1992) Respiratory metabolism and gene expression during seed germination. *Plant Growth Regulation* **11**, 211–224.

Bradford, K.J., Chen, F., Cooley, M.B., Dahal, P., Downie, B., Fukunaga, K.K., Gee, O.H., Gurusinghe, S., Mella, R.A., Nonogaki, H., Wu, C-T., Yang, H. and Kim, K-O. (2000) Gene expression prior to radicle emergence in imbibed tomato seeds. pp. 231–251 *in* Black, M.; Bradford, K.J.; Vázquez-Ramos, J. (Eds) *Seed biology. Advances and applications*. Wallingford, CABI Publishing.

Briggs, D.E. and McGuinness, G. (1992) Microbes on barley grains. *Journal of the Institute of Brewing* **98**, 249–255.

Brooks, C.A., Yu, K.S. and Mitchell, C.A. (1985) Salicylhydroxamic acid potentiates germination of 'Waldmann's Green' lettuce seed. *Plant Physiology* **79**, 386–388.

Brooks, S.P.J. and Storey, K.B. (1997) Glycolytic controls in estivation and anoxia: a comparison of metabolic arrest in land and marine molluscs. *Comparative Biochemistry and Physiology* **118A**, 1103–1114.

Busa, W.B. and Nuccitelli, R. (1984) Metabolic regulation via intracellular pH. *American Journal of Physiology* **246**, R409–R438.

Cáceres, C.E. (1997) Dormancy in invertebrates. *Invertebrate Biology* **116**, 371–383.

Cairns, A.L.P. and De Villiers, O.T. (1986) Breaking dormancy of *Avena fatua* L. seed by treatment with ammonia. *Weed Research* **26**, 191–197.

Charbonneau, M. and Grandin, N. (1989) The egg of *Xenopus laevis*: a model system for studying cell activation. *Cell Differentiation and Development* **28**, 71–94.

Clark, J.F. (1898) Electrolytic dissociation and toxic effect. *Journal of Physical Chemistry* **3**, 263–316.

Clegg, J.S., Drinkwater, L.E. and Sorgeloos, P. (1996) The metabolic status of diapause embryos of *Artemia franciscana* (SFB). *Physiological Zoology* **69**, 49–66.

Cohn, M.A. (1987) Mechanisms of physiological seed dormancy. pp. 14–20 *in* Frasier, G.W.; Evans, R.A. (Eds) *Seed and seedbed ecology of rangeland plants*. Washington DC, USDA-ARS.

Cohn, M.A. (1989) Factors influencing the efficacy of dormancy-breaking chemicals. pp. 261–267 *in* Taylorson, R.B. (Ed.) *Recent advances in the development and germination of seeds*. New York, Plenum Press.

Cohn, M.A. (1996a) Chemical mechanisms of breaking seed dormancy. *Seed Science Research* **6**, 95–99.

Cohn, M.A. (1996b) Operational and philosophical decisions in seed dormancy research. *Seed Science Research* **6**, 147–153.

Cohn M.A. (1997) QSAR modelling of dormancy-breaking chemicals. pp. 289–295 *in* Ellis, R.H.; Black, M.; Murdoch, A.J.; Hong, T.D. (Eds) *Basic and applied aspects of seed biology*. Dordrecht, Kluewer Academic.

Cohn, M.A. and Castle, L. (1984) Dormancy in red rice. IV. Response of unimbibed and imbibing seeds to nitrogen dioxide. *Physiologia Plantarum* **60**, 552–556.

Cohn, M.A. and Footitt, S. (1993) Initial signal transduction steps during the dormancy-breaking process. pp. 599–605 *in* Côme, D.; Corbineau, F. (Eds) *Proceedings of the fourth international workshop on seeds: basic and applied aspects of seed biology*. *Volume 2*. Paris, ASFIS.

Cohn, M.A. and Hilhorst, H.W.M. (2000) Alcohols that break seed dormancy: The anaesthetic hypothesis, dead or alive? pp. 259–274 *in* Viémont, J.-D.; Crabbé, J. (Eds) *Dormancy in plants. From whole plant behaviour to cellular control*. Wallingford, CABI Publishing.

Cohn, M.A. and Hughes, J.A. (1986) Seed dormancy in red rice. V. Response to azide, hydroxylamine, and cyanide. *Plant Physiology* **80**, 531–533.

Cohn, M.A., Butera, D.L. and Hughes, J.A. (1983) Seed dormancy in red rice. III. Response to nitrite, nitrate, and ammonium ions. *Plant Physiology* **73**, 381–384.

Cohn, M.A., Chiles, L.A., Hughes, J.A. and Boullion, K.J. (1987) Seed dormancy in red rice. VI. Monocarboxylic acids: a new class of pH-dependent germination stimulants. *Plant Physiology* **84**, 716–719.

Cohn, M.A., Jones, K.L., Chiles, L.A. and Church, D.F. (1989) Seed dormancy in red rice. VII. Structure–activity studies of germination stimulants. *Plant Physiology* **89**, 879–882.

Cohn, M.A., Church, D.F., Ranken, J. and Sanchez, V. (1991) Hydroxyl group position governs activity of dormancy-breaking chemicals. *Plant Physiology* **96**, S-63.

Côme, D., Corbineau, F. and Lecat, S. (1988) Some aspects of metabolic regulation of cereal seed germination and dormancy. *Seed Science and Technology* **16**, 175–186.

Corbineau, F., Gouble, B., Lecat, S. and Côme, D. (1991) Stimulation of germination of dormant oat (*Avena sativa* L.) seeds by ethanol and other alcohols. *Seed Science Research* **1**, 21–28.

Datta, T. (1979) Effect of organic and inorganic compounds and carbon dioxide in the excystment of soil amoebae. *Archiv für Protistenkunde* **121**, 155–161.

Dedonder, A., Rethy, R., Fredericq, H. and De Greef, J.A. (1992) Phytochrome-mediated changes in the ATP content of *Kalanchoë blossfeldiana* seeds. *Plant Cell and Environment* **15**, 479–484.

De Greef, J.A., Fredericq, H., Rethy, R., Dedonder, A., De Petter, E. and Van Wiemeersch, L. (1989) Factors eliciting the germination of photoblastic Kalanchoë seeds. pp. 241–260 *in* Taylorson, R.B. (Ed.) *Recent advances in the development and germination of seeds*. London, Plenum Press.

Denlinger, D.L., Campbell, J.J. and Bradfield, J.Y. (1980) Stimulatory effect of organic solvents on initiating development in diapausing pupae of the flesh fly, *Sarcophaga crassipalpis*, and the tobacco hornworm, *Manduca sexta*. *Physiological Entomology* **5**, 7–15.

Drinkwater, L.E. and Crowe, J.H. (1987) Regulation of embryonic diapause in *Artemia*: Environmental and physiological signals. *Journal of Experimental Zoology* **241**, 297–307.

Dyer, W.E. (1993) Dormancy-associated embryonic mRNAs and proteins in imbibing *Avena fatua* caryopses. *Physiologia Plantarum* **88**, 201–211.

Eckerson, S. (1913) A physiological and chemical study of after-ripening. *Botanical Gazette* **55**, 286–299.

Epel, D. (1989) Arousal of activity in sea urchin eggs at fertilization. pp. 361–385 *in* Schatten, H.; Schatten, G. (Eds) *The cell biology of fertilization*. San Diego, Academic Press.

Epel, D. (1990) The initiation of development at fertilization. *Cell Differentiation and Development* **29**, 1–12.

Esashi, Y., Kusuyama, K., Tazaki, S. and Ishihara, N. (1981a) Necessity of a balance between CN-sensitive and CN-resistant respirations for germination of cocklebur seeds. *Plant and Cell Physiology* **22**, 65–71.

Esashi, Y., Sakai, Y. and Ushizawa, R. (1981b) Cyanide-sensitive and cyanide-resistant respiration in the germination of cocklebur seeds. *Plant Physiology* **67**, 503–508.

Fontaine, O., Huault, C., Pavis, N. and Billard, J.P. (1994) Dormancy breakage of *Hordeum vulgare* seeds: effects of hydrogen peroxide and scarification on glutathione level and glutathione reductase activity. *Plant Physiology and Biochemistry* **32**, 677–683.

Footitt, S. and Cohn, M.A. (1992) Seed dormancy in red rice. VIII. Embryo acidification during dormancy-breaking and subsequent germination. *Plant Physiology* **100**, 1196–1202.

Footitt, S. and Cohn, M.A. (1995) Seed dormancy in red rice. IX. Embryo fructose 2,6-bisphosphate during dormancy-breaking and subsequent germination. *Plant Physiology* **107**, 1365–1370.

Footitt, S., Vargas, D. and Cohn, M.A. (1995) Seed dormancy in red rice. X. A ^{13}C NMR study of the metabolism of dormancy-breaking chemicals. *Physiologia Plantarum* **94**, 667–671.

Freeman, G. and Ridgway, E.B. (1993) The role of intracellular calcium and pH during fertilization and egg activation in the hydrozoan *Phialidium*. *Developmental Biology* **156**, 176–190.

French, R.C. (1984) Stimulation of uredospore germination of *Puccinia helianthi* and *Uromyces vignae* by aromatic nitriles and related flavorlike compounds. *Journal of Agricultural and Food Chemistry* **32**, 556–561.

French, R.C. and Leather, G.R. (1979) Screening of nonanal and related volatile flavor compounds on the germination of 18 species of weed seed. *Journal of Agricultural and Food Chemistry* **27**, 828–832.

French, R.C., Kujawski, P.T. and Leather, G.R. (1986) Effect of various flavor-related compounds on germination of curly dock seed (*Rumex crispus*) and curly dock rust (*Uromyces rumicis*). *Weed Science* **34**, 398–402.

Garciarrubio, A., Legaria, J.P. and Covarrubias, A.A. (1997) Abscisic acid inhibits germination of mature *Arabidopsis* seeds by limiting the availability of energy and nutrients. *Planta* **203**, 182–187.

Gehring, C.A., Irving, H.R. and Parish, R.W. (1990) Effects of auxin and abscisic acid on cytosolic calcium and pH in plant cells. *Proceedings of the National Academy of Sciences, USA* **87**, 9645–9649.

Gendraud, M. and Lafleuriel, J. (1983) Caractéristiques de l'absorption du saccharose et du tétraphényl-phosphonium par les parenchymes de tubercules de Topinambour, dormants et non dormants, cultivés *in vitro*. *Physiologie Vegetale* **21**, 1125–1133.

Goldmark, P.J., Curry, J., Morris C.F. and Walker-Simmons, M.K. (1992) Cloning and expression of an embryo-specific mRNA up-regulated in hydrated dormant seeds. *Plant Molecular Biology* **19**, 433–441.

Gómez-Cadenas, A., Verhey, S.D., Holappa, L.D., Shen, Q., Ho, T-H.D. and Walker-Simmons, M.K. (1999) An abscisic acid-induced protein kinase, PKABA1, mediates abscisic acid-suppressed gene expression in barley aleurone layers. *Proceedings of the National Academy of Sciences, USA* **96**, 1767–1772.

Gould, M.C. and Stephano, J.L. (1993) Nuclear and cytoplasmic pH increase at fertilization in *Urechis caupo*. *Developmental Biology* **159**, 608–617.

Grainger, J.L., Winkler, M.M., Shen, S.S. and Steinhardt, R.A. (1979) Intracellular pH controls protein synthesis rate in the sea urchin egg and early embryo. *Developmental Biology* **68**, 396–406.

Guppy, M. and Withers, P. (1999) Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biological Review* **74**, 1–40.

Guppy, M., Fuery, C.J. and Flanigan, J.E. (1994) Biochemical principals of metabolic depression. *Comparative Biochemistry and Physiology* **109B**, 175–189.

Hance, B.A. and Bevington, J.M. (1992) Changes in protein synthesis during stratification and dormancy release in embryos of sugar maple (*Acer saccharum*). *Physiologia Plantarum* **86**, 365–371.

Hand, S.C. (1991) Metabolic dormancy in aquatic invertebrates. *Advances in Comparative and Environmental Physiology* **8**, 1–50.

Hand, S.C. and Gnaiger, E. (1988) Anaerobic dormancy quantified in *Artemia* embryos: a calorimetric test of the control mechanism. *Science* **239**, 1425–1427.

Hand, S.C. and Hardewig, I. (1996) Downregulation of cellular metabolism during environmental stress: mechanisms and implications. *Annual Review of Physiology* **58**, 539–563.

Hand, S.C. and Podrabsky, J.E. (2000) Bioenergetics of diapause and quiescence in aquatic animals. *Thermochimica Acta* **349**, 31–42.

Harding, D. (1951) Initiation of cell division in the arbacia egg by injury substances. *Physiological Zoology* **24**, 54–69.

Hareland, G.A. and Madson, M.A. (1989) Barley dormancy and fatty acid composition of lipids isolated from freshly harvested and stored kernels. *Journal of the Institute of Brewing* **95**, 437–442.

Harman, G.E., Mattick, L.R., Nash, G. and Nedrow, B.L. (1980) Stimulation of fungal spore germination and inhibition of sporulation in fungal vegetative thalli by fatty acids and their volatile peroxidation products. *Canadian Journal of Botany* **58**, 1541–1547.

Harvey, E.N. (1911) Studies on the permeability of cells. *Journal of Experimental Zoology* **10**, 507–556.

Heimovaara-Dijkstra, S., Mundy, J. and Wang, M. (1995) The effect of intracellular pH on the regulation of the Rab 16A and the α-amylase 1/6–4 promoter by abscisic acid and gibberellin. *Plant Molecular Biology* **27**, 815–820.

Hendricks, S.B. and Taylorson, R.B. (1974) Promotion of seed germination by nitrate, nitrite, hydroxylamine, and ammonium salts. *Plant Physiology* **54**, 304–309.

Henis, Y. (1987) *Survival and dormancy of microorganisms*. New York, John Wiley & Sons.

Hervé, M., Goudeau, M., Neumann, J.M., Debouzy, J.C. and Goudeau, H. (1989) Measurement of an intracellular pH rise after fertilization in crab eggs using ^{31}P-NMR. *European Biophysics Journal* **17**, 191–199.

Hilhorst, H.W.M. (1995) A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research* **5**, 61–73.

Hilhorst, H.W.M. (1998) The regulation of secondary dormancy. The membrane hypothesis revisited. *Seed Science Research* **8**, 77–90.

Hiroe, M. and Inoh, S. (1954) Artificial parthenogenesis in *Sargassum piluliferum* C. AG. *Botanical Magazine* **67**, 271–274.

Hochachka, P.W. and Guppy, M. (1987) *Metabolic arrest and the control of biological time*. Cambridge, Massachusetts, Harvard University Press.

Hoecker, U., Vasil, I.K. and McCarty, D.R. (1995) Integrated control of seed maturation and germination programs by activator and repressor functions of Viviparous-1 of maize. *Genes and Development* **9**, 2459–2469.

Holdsworth, M., Kurup, S. and McKibbin, R. (1999) Molecular and genetic mechanisms regulating the transition from embryo development to germination. *Trends in Plant Science* **4**, 275–280.

Ii, I. and Rebhun, L.I. (1982) Release of glucose-6-phosphate dehydrogenase from cortex of *Spisula* eggs at fertilization and its recombination after meiosis. *Developmental Biology* **91**, 171–182.

Isfort, R.J., Cody, D.B., Asquith, T.N., Ridder, G.M., Stuard, S.B. and Leboeuf, R.A. (1993) Induction of protein phosphorylation, protein synthesis, immediate-early-gene expression and cellular proliferation by intracellular pH modulation. Implications for the role of hydrogen ions in signal transduction. *European Journal of Biochemistry* **213**, 349–357.

Isfort, R.J., Stuard, S.B., Cody, D.B., Ridder, G.M. and Leboeuf, R.A. (1995) Modulation of the platelet-derived-growth-factor-induced calcium signal by extracellular/intracellular pH in Syrian hamster embryo cells. Implication for the role of calcium in mitogenic signaling. *European Journal of Biochemistry* **234**, 801–810.

Isono, N. (1963) Carbohydrate metabolism in sea urchin eggs IV. Intracellular localization of enzymes of pentose phosphate cycle in unfertilized and fertilized eggs. *Journal of the Faculty of Science of the University of Tokyo, Section IV* **10**, 37–53.

Isono, N., Tsusaka, A. and Nakano, E. (1963) Studies on glucose-6-phosphate dehydrogenase in sea urchin eggs I. *Journal of the Faculty of Science of the University of Tokyo, Section IV* **10**, 55–66.

Jeffries, W.B. (1956) Studies on excystment in the hypotrichous ciliate *Pleurotricha lanceolata*. *Journal of Protozoology* **3**, 136–144.

Jeffries, W.B. (1962) Studies on specific chemicals as excysting agents for *Pleurotricha lanceolata*. *Journal of Protozoology* **4**, 375–376.

Jensen, J.B., Nyberg, P.A., Burton, S.D. and Jolley, W.R. (1976) The effects of selected gases on excystation of coccidian oocysts. *Journal of Parasitology* **62**, 195–198.

Johnson, J.D., Epel, D. and Paul, M. (1976) Intracellular pH and activation of sea urchin eggs after fertilisation. *Nature* **262**, 661–664.

Johnson, R.R., Cranston, H.J., Chaverra, M.E. and Dyer, W.E. (1995) Characterization of cDNA clones for differentially expressed genes in embryos of dormant and nondormant *Avena fatua* L. caryopses. *Plant Molecular Biology* **28**, 113–122.

Jones, H.A. (1920) Physiological study of maple seeds. *Botanical Gazette* **69**, 127–152.

Jones, H.D., Peters, N.C.B. and Holdsworth, M.J. (1997) Genotype and environment interact to control dormancy and differential expression of the *VIVIPAROUS* 1 homologue in embryos of *Avena fatua*. *Plant Journal* **12**, 911–920.

Jones, H.D., Kurup, S., Peters, N.C.B. and Holdsworth, M.J. (2000) Identification and analysis of proteins that interact with the *Avena fatua* homologue of the maize transcription factor VIVIPAROUS 1. *Plant Journal* **21**, 133–142.

Jungreis, A.M. (1978) Insect dormancy. pp. 47–112 *in* Clutter, M.E. (Ed.) *Dormancy and developmental arrest*. London, Academic Press.

Kirillova, I.P., Agre, N.S. and Kalakutskii, L.V. (1974) Control of the emergence of *Thermoactinomyces vulgaris* endospores from dormancy. *Microbiology* **43**, 894–898.

Koornneef, M., Leon-Kloosterziel, K.M., Schwartz, S.H. and Zeevaart, J.A.D. (1998) The genetic and molecular dissection of abscisic acid biosynthesis and signal transduction in *Arabidopsis*. *Plant Physiology and Biochemistry* **36**, 83–89.

Kowalczyk, S. (1989) Rapid oscillation of fructose 2,6-bisphosphate levels in plant storage tissues as a result of resumption of metabolic activity. *Biochemie und Physiologie der Pflanzen* **184**, 371–376.

Larondelle, Y., Corbineau, F., Dethier, M., Côme, D. and Hers, H.G. (1987) Fructose 2,6-bisphosphate in germinating oat seeds. A biochemical study of seed dormancy. *European Journal of Biochemistry* **166**, 605–610.

Leather, G.R., Sung, S.J. and Hale, M.G. (1992) The wounding response of dormant barnyardgrass (*Echinochloa crus-galli*) seeds. *Weed Science* **40**, 200–203.

Lenoir, C., Corbineau, F. and Côme D. (1986) Barley (*Hordeum vulgare)* seed dormancy as related to glumella characteristics. *Physiologia Plantarum* **68**, 301–307.

LePage-Degivry, M.T. and Garello, G. (1992) *In situ* abscisic acid synthesis. A requirement for induction of embryo dormancy in *Helianthus annuus*. *Plant Physiology* **98**, 1386–1390.

Levinson, H.S. and Sevag, M.G. (1953) Stimulation of germination and respiration of the spores of *Bacillus megaterium* by manganese and monovalent anions. *Journal of General Physiology* **36**, 617–629.

Li, B. and Foley, M.E. (1995) Cloning and characterization of differentially expressed genes in imbibed dormant and afterripened *Avena fatua* embryos. *Plant Molecular Biology* **29**, 823–831.

Li, B. and Foley, M.E. (1996) Transcriptional and posttranscriptional regulation of dormancy-associated gene expression by afterripening in wild oat. *Plant Physiology* **110**, 1267–1273.

Lillie, R.S. (1910) Physiology of cell-division. II. The action of isotonic solutions of neutral salts on unfertilized eggs of asterias and arbacia. *American Journal of Physiology* **26**, 106–133.

Lillie, R.S. (1913) The physiology of cell division. V. Substitution of anesthetics for hypertonic sea-water and cyanide in artificial parthenogenesis in starfish eggs. *Journal of Experimental Zoology* **15**, 23–47.

Lillie, R.S. (1926) The activation of starfish eggs by acids. *Journal of General Physiology* **8**, 339–367.

Lillie, R.S. (1927) The activation of starfish eggs by acids. II. The action of substituted benzoic acids and of benzoic and salicylic acids as influenced by their salts. *Journal of General Physiology* **10**, 703–723.

Loeb, J. (1913) *Artificial parthenogenesis and fertilization*. Chicago, University of Chicago Press.

Loomis, S.H., Hand, S.C. and Fell, P.E. (1996) Metabolism of gemmules from the freshwater sponge *Eunapius fragilis* during diapause and post-diapause states. *Biological Bulletin* **191**, 385–392.

Lyon, E.P. (1903) Experiments in artificial parthenogenesis. *American Journal of Physiology* **9**, 308–318.

Major, W. and Roberts, E.H. (1968) Dormancy in cereals. I. The effects of oxygen and respiratory inhibitors. *Journal of Experimental Botany* **19**, 77–89.

Matsumori, K., Izumi, S. and Watanabe, H. (1989) Hormone-like action of 3-octanol and 1-octen-3-ol from *Botrytis cinerea* on the pine wood nematode, *Bursaphelenchus xylophilus*. *Agricultural and Biological Chemistry* **53**, 1777–1781.

Mayer, A.M. and Evenari, M. (1953) The activity of organic acids as germination inhibitors and its relation to pH. *Journal of Experimental Botany* **4**, 257–263.

McCarty, D.R. (1995) Genetic control and integration of maturation and germination pathways in seed development. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**, 71–93.

Mertens, E. (1991) Pyrophosphate-dependent phospho-fructokinase, an anaerobic glycolytic enzyme? *FEBS Letters* **285**, 1–-5.

Mertens, E., Larondelle, Y. and Hers, H.G. (1990) Induction of pyrophosphate:fructose 6-phosphate 1-phosphotransferase by anoxia in rice seedlings. *Plant Physiology* **93**, 584–587.

Miller, B.S. and Epel, D. (1999) The roles of changes in NADPH and pH during fertilization and artificial activation of the sea urchin egg. *Developmental Biology* **216**, 394–405.

Moorhead, G.B.G. and Plaxton, W.C. (1988) Binding of glycolytic enzymes to a particulate fraction in carrot and sugar beet storage roots. Dependence on metabolic state. *Plant Physiology* **86**, 348–351.

Morris, C.F., Anderberg, R.J., Goldmark, P.J. and Walker-Simmons, M.K. (1991) Molecular cloning and expression of abscisic acid-responsive genes in embryos of dormant wheat seeds. *Plant Physiology* **95**, 814–821.

Myers, S.P., Foley, M.E. and Nichols, M.B. (1997) Developmental differences between germinating after-ripened and dormant excised *Avena fatua* L. embryos. *Annals of Botany* **79**, 19–23.

Nuccitelli, R., Webb, D.J., Lagier, S.T. and Matson, G.B. (1981) ^{31}P-NMR reveals increased intracellular pH after fertilization in *Xenopus* eggs. *Proceedings of the National Academy of Sciences, USA* **78**, 4421–4425.

Nyberg, P.A., Bauer, D.H. and Knapp, S.E. (1968) Carbon dioxide as the initial stimulus for excystation of *Eimeria tenella* oocysts. *Journal of Protozoology* **15**, 144–148.

Ohta, A. (1988) Effects of butyric acid and related compounds on basidiospore germination of some mycorrhizal fungi. *Transactions of the Mycological Society of Japan* **29**, 375–381.

Overton, C.E. (1901) Studien über die narkose. G Fischer, Jena. *in* Lipnick, R.L. (Ed.) Translated as *Studies of narcosis. Charles Ernest Overton.* (1991). New York, Chapman and Hall.

Overton, J.B. (1913) Artificial parthenogenesis in fucus. *Science* **37**, 841–844.

Pack, D.A. (1921) After-ripening and germination of *Juniperus* seeds. *Botanical Gazette* **71**, 32–60.

Paek, N.C., Lee, B.-M., Bai, D.G. and Smith, J.D. (1998) Inhibition of germination gene expression by Viviparous-1 and ABA during maize kernal development. *Molecules and Cells* **8**, 336–342.

Palevitch, D. and Thomas, T.H. (1976) Enhancement by low pH of gibberellin effects on dormant celery seeds and embryoless half-seeds of barley. *Physiologia Plantarum* **37**, 247–252.

Pepper, J.H. (1937) Breaking the dormancy in the sugar-beet webworm, *L. sticticalis* L., by means of chemicals. *Journal of Economic Entomology* **30**, 380.

Petronijevic, T. and Rogers, W.P. (1987a) Undissociated bases as the stimulus for the development of early parasitic stages of nematodes. *International Journal of Parasitology* **17**, 911–915.

Petronijevic, T. and Rogers, W.P. (1987b) The physiology of infection with nematodes: The role of intracellular pH in the development of the early parasitic stage. *Comparative Biochemistry and Physiology* **88A**, 207–212.

Petronijevic, T., Rogers, W.P. and Sommerville, R.I. (1986) Organic and inorganic acids as the stimulus for exsheathment of infective juveniles of nematodes. *International Journal of Parasitology* **16**, 163–168.

Preston, R.A. and Douthit, H.A. (1988) Functional relationships between L- and D-alanine, inosine, and NH_4Cl during germination of spores of *Bacillus cereus* T. *Journal of General Microbiology* **134**, 3001–3010.

Rees, B.B., Swezey, R.R., Kibak, H. and Epel, D. (1996) Regulation of the pentose shunt pathway at fertilization in sea urchin eggs. *Invertebrate Reproduction and Development* **30**, 123–134.

Renfree, M.B. (1978) Embryonic diapause in mammals – a developmental strategy. pp. 1–46 *in* Clutter, M.E. (Ed.) *Dormancy and developmental arrest*. London, Academic Press.

Ritchie, S., Swanson, S.J. and Gilroy, S. (2000) Physiology of the aleurone layer and starchy endosperm during grain development and early seedling growth: new insights from cell and molecular biology. *Seed Science Research* **10**, 193–212.

Roberts, E.H. and Smith, R.D. (1977) Dormancy and the pentose phosphate pathway. pp. 385–408 *in* Khan, A. (Ed.) *The physiology and biochemistry of seed dormancy and germination*. Amsterdam, Elsevier/NorthHolland.

Rode, L.J. and Foster, J.W. (1961) Physiological and chemical germination of spores of *Bacillus megaterium*. *Zeitschrift für Allgemeine Mikrobiologie* **1**, 307–322.

Rode, L.J. and Foster, J.W. (1965) Gaseous hydrocarbons and the germination of bacterial spores. *Proceedings of the National Academy of Sciences, USA* **53**, 31–38.

Rose, R.C. (1919) After-ripening and germination of seeds of *Tilia, Sambuca,* and *Rubus*. *Botanical Gazette* **67**, 281–308.

Schaefer, F.W., Rice, E.W. and Hoff, J.C. (1984) Factors promoting *in vitro* excystation of *Giardia muris* cysts. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **78**, 795–800.

Schonbeck, M.W. and Egley, G.H. (1980) Effects of temperature, water potential, and light on germination responses of redroot pigweed seeds to ethylene. *Plant Physiology* **65**, 1149–1154.

Sibilia, C. (1930) La germinazione delle teleutospore di *Puccinia graminis* e *P. triticina*. *Bollettino della Regia Stazione di Patalogia Vegetale* **10**, 164–190.

Simpson, G.M. (1990) *Seed dormancy in grasses*. Cambridge, Cambridge University Press.

Slifer, E.H. (1946) The effects of xylol and other solvents on diapause in the grasshopper egg; together with a possible explanation for the action of these agents. *Journal of Experimental Zoology* **102**, 333–356.

Stitt, M. (1990) Fructose-2,6-bisphosphate as a regulatory molecule in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**, 153–185.

Sussex, I.M. (1978) Dormancy and development. pp. 297–301 *in* Clutter, M.E. (Ed.) *Dormancy and developmental arrest*. London, Academic Press.

Sussman, A.S. and Halvorson, H.O. (1966) *Spores, their dormancy and germination*. New York, Harper and Row.

Swezey, R.R. and Epel, D. (1986) Regulation of glucose-6-phosphate dehydrogenase activity in sea urchin eggs by reversible association with cell structural elements. *Journal of Cell Biology* **103**, 1509–1515.

Swezey, R.R. and Epel, D. (1995) The *in vivo* rate of glucose-6-phosphate dehydrogenase activity in sea urchin eggs determined with a photolabile caged substrate. *Developmental Biology* **169**, 733–744.

Taylorson, R.B. (1979) Response of weed seeds to ethylene and related hydrocarbons. *Weed Science* **27**, 7–10.

Taylorson, R.B. (1980) Aspects of seed dormancy in fall panicum (*Panicum dichotomiflorum*). *Weed Science* **28**, 64–67.

Taylorson, R.B. (1984) Prevention of action of far-red-absorbing phytochrome in *Rumex crispus* L. seeds by ethanol. *Plant Physiology* **74**, 223–226.

Taylorson, R.B. (1988) Anaesthetic enhancement of *Echinochloa crus-galli* (L.) Beauv. seed germination: possible membrane involvement. *Journal of Experimental Botany* **39**, 50–58.

Taylorson, R.B. (1989) Response of redroot pigweed (*Amaranthus retroflexus*) and witchgrass (*Panicum capillare*) seeds to anesthetics. *Weed Science* **37**, 93–97.

Taylorson, R.B. and Hendricks, S.B. (1979) Overcoming dormancy in seeds with ethanol and other anesthetics. *Planta* **145**, 507–510.

Taylorson, R.B. and Hendricks, S.B. (1980/81) Anesthetic release of seed dormancy – an overview. *Israel Journal of Botany* **29**, 273–280.

Thevelein, J.M., Van Assche, J.A., Carlier, A.R. and Heremans, K. (1979) Heat activation of *Phycomyces blakesleeanus* spores: thermodynamics and effect of alcohols, furfural, and high pressure. *Journal of Bacteriology* **139**, 478–485.

Thevelein, J.M., Van Assche, J.A. and Carlier, A.R. (1983) Isopropyl-substituted phenols have a different effect from other phenols on the breaking of dormancy by heat shock in *Phycomyces blakesleeanus* spores. *Journal of General Microbiology* **129**, 727–733.

Toole, V.K. and Cathey, H.M. (1961) Responses to gibberellin of light-requiring seeds of lettuce and *Lepidium virginicum*. *Plant Physiology* **36**, 663–671.

Tseng, S. (1964) Breaking dormancy of rice seed with carbon dioxide. *Proceedings of the International Seed Testing Association* **29**, 445–450.

Van Beckum, J.M.M., Libbenga, K.R. and Wang, M. (1993) Abscisic acid and gibberellic acid-regulated responses of embryos and aleurone layers isolated from dormant and nondormant barley grains. *Physiologia Plantarum* **89**, 483–489.

Van der Veen, R., Heimovaara-Dijkstra, S. and Wang M. (1992) Cytosolic alkalinization mediated by abscisic acid is necessary, but not sufficient, for abscisic acid-induced gene expression in barley aleurone protoplasts. *Plant Physiology* **100**, 699–705.

Van Laere, A., Van Schaftingen, E. and Hers, H.G. (1983) Fructose 2,6-bisphosphate and germination of fungal spores. *Proceedings of the National Academy of Sciences, USA* **80**, 6601–6605.

Van Mulders, R.M., Van Laere, A.J. and Verbeke, M.N. (1986) Effects of pH and cations on the germination induction of *Phycomyces* spores with carboxylic acids. *Biochemie und Physiologie der Pflanzen* **181**, 103–115.

Van Schaftingen, E. (1987) Fructose 2,6-bisphosphate. *Advances in Enzymology and Related Areas of Molecular Biology* **59**, 315–395.

Van Schaftingen, E. and Hers, H.G. (1983) Fructose 2,6-bisphosphate in relation with the resumption of metabolic activity in slices of Jerusalem artichoke tubers. *FEBS Letters* **164**, 195–200.

Wadsworth, W.G. and Riddle, D.L. (1988) Acidic intracellular pH shift during *Caenorhabditis elegans* larval development. *Proceedings of the National Academy of Sciences, USA* **85**, 8435–8438.

Walker-Simmons, M.K. (1998) Protein kinases in seeds. *Seed Science Research* **8**, 193–200.

Walker-Simmons, M.K. (2000) Recent advances in ABA-regulated gene expression in cereal seeds: Evidence for regulation by PKABA1 protein kinase. pp. 271–276 *in* Black, M.; Bradford, K.J.; Vázquez-Ramos, J. (Eds) *Seed biology. Advances and applications*. Wallingford, CABI Publishing.

Whitaker, M.J. and Steinhardt, R.A. (1982) Ionic regulation of egg activation. *Quarterly Reviews of Biophysics* **15**, 593–666.

Zagorski, S. and Lewak, S. (1984) Are effects of gibberellic and abscisic acids on lettuce seed germination pH-dependent? *Acta Physiologia Plantarum* **6**, 27–32.

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