Using the power of developmental biology for drug discovery

Simon J. Rhodes and Rosamund C. Smith

With the rapid advancement of the human genome project, the race is on to identify the relatively few useful target genes for drug discovery. The power of developmental biology in this effort has recently begun to be recognized. In addition to the use of model developmental organisms for functional studies, certain pluripotent cell populations and gene products that are present during embryogenesis are candidate therapeutic tools for use in the treatment of human diseases. These agents could be used to repair diseased tissues by inducing or directing developmental programs that recapitulate embryonic processes to replace specialized cells. The direct manipulation of embryonic development by pharmaceutical agents is a unique application of developmental biology that is currently being explored in animal agriculture.

In the postgenomic era of drug discovery for human and animal health applications, emphasis is on the determination of the function of genes, their possible association with disease or animal production traits, and their identification and validation as targets for drug discovery. This review focuses on the emerging use of developmental biology in drug discovery and examines four main areas.

- The use of the developmental programs of model organisms as test systems to determine gene function or gene interactions.
- The use of embryonic proteins as therapeutic agents.
- The use of stem cells as therapeutic agents.
- The animal embryo as a direct target for drug action in agriculture.

Postgenomic drug discovery

It is estimated that there are 80,000–100,000 genes in the human genome. Currently, drug companies are working with about 500 'targets' or gene products, but it has been estimated that the sequencing of the human genome will boost this number by an order of magnitude, so it can be expected that 3000–10,000 targets will become available within the next few years[1]. The key question is how rare target genes can be quickly identified from the pool of all genes. Recently, much effort has gone into technologies that analyze the expression patterns of thousands of gene transcripts at one time, allowing for comparison of gene expression between normal and diseased states. These technologies include expressed sequence tags[2], serial analysis of gene expression[3], and microarrays[4,5]. Information generated using such technologies is becoming increasingly available, as exemplified by the Cancer Genome Anatomy Project (CGAP) Web site (www.ncbi.nlm.nih.gov/ncicgap), which is a complete database of the known active and silent genes in normal, precancerous and tumor cells[6]. Using a similar strategy, the study of proteomics focuses on the analysis of protein expression patterns in normal and diseased states, with

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the assumption that target gene products are likely to change expression under disease conditions[7]. However, with both RNA and protein expression approaches, a disease connection alone is not usually sufficient for the identification and validation of a gene as a target for drug discovery. It is necessary to have some information about the function of the gene in order to know whether the disease association is causal or not[8]. Functional genomics focuses on this issue and uses a variety of approaches to ascertain the function of genes, including the analysis of overexpression, underexpression and knockout phenotypes in cell culture or in transgenic mice. Complementary to this, sequence database mining and comparison searches in the area of bioinformatics are becoming increasingly important by identifying sequence similarities between novel genes and genes of known function, and through the identification of recognizable functional motifs within putative target genes[9].

Model organisms in functional genomics

The conservation of gene sequence and function between widely divergent animal species allows the use of simple, easily accessible organisms to probe gene function[10]. The model organisms of choice for these functional studies are the species most often used in the field of developmental biology; namely, the mouse (*Mus musculus*), the chick (*Gallus gallus domesticus*), the frog (*Xenopus laevis*), the zebrafish (*Brachydanio rerio*), the fruit fly (*Drosophila melanogaster*), the nematode worm (*Caenorhabditis elegans*) and unicellular yeast (*Saccharomyces cerevisiae*). Plans for the complete sequencing of the genomes of *Drosophila* and *C. elegans* make these two organisms particularly useful. The *C. elegans* sequence is already over 70% complete (http://www.sanger.ac.uk/Projects/C_elegans/), and, although the *Drosophila* genome is only 5–10% sequenced, it is expected to be completed in 5–7 years (http://fruitfly.berkeley.edu/BDGP/genomic_info.html). The zebrafish has become a favorable model organism and can be expected to have a major impact in the future as more tools and information accumulate[11]. Notably, a comprehensive screen for mutations in genes affecting development of this vertebrate has recently been accomplished[12]. Evidence for the utility of the model organism approach to functional genomics comes from work where known human disease genes have been searched for in model organisms. It was found that the majority of known disease genes have counterparts in lower species. The most homologies were found for *C. elegans*, probably because of the extensive sequence information that has already accumulated for the genome of this organism[13,14]. Specific databases and search tools to look for orthologs of novel genes in model organisms are now available[13]. A summary of the advantages and disadvantages of each of the model organisms is summarized in Table 1. Although no one organism is suitable for all studies – each has strengths and weaknesses – in total they make very powerful tools for gene function studies.

Table 1. Advantages and disadvantages of model organisms used in developmental biology based drug discovery

Organism	Advantages	Disadvantages
Mouse (*Mus musculus*)	Mammal, some genetics, transgenics, knockouts, some genome data	Costly, long generation time
Chicken (*Gallus gallus domesticus*)	Vertebrate, easily manipulated	No traditional genetics, sparse genome data
African clawed frog (*Xenopus laevis*)	Vertebrate, easily manipulated, transgenics	No traditional genetics, sparse genome data
Zebrafish (*Brachydanio rerio*)	Vertebrate, easily manipulated, genetics	Sparse genome data
Nematode worm (*Caenorhabditis elegans*)	Genetics, some genome data	Invertebrate
Fruit fly (*Drosophila melanogaster*)	Genetics, some genome data	Invertebrate
Yeast (*Saccharomyces cerevisiae*)	Genetics, complete genome data	Unicellular

Functional studies in model organisms

The power of genetic or physical manipulation and ease of overexpression or underexpression of genes of interest in these organisms allow functional studies to be conducted that can shed light on the function of a gene in a short period of time. It is possible to express a candidate human gene directly in the model organism or to work with its ortholog identified by sequence similarity. Functional homology can be tested, particularly in those species amenable to genetics such as *Drosophila* and *C. elegans*, by

examining the ability of the human gene to rescue mutants of the fly or worm counterpart[15,16]. The genetic pathway of a particular gene of interest can be identified by looking for suppressors or enhancers of mutant phenotypes. If a particular disease is a consequence of a causal mutation in a gene that cannot be overcome, it becomes necessary to activate or inactivate the genetic pathway downstream of the mutant gene as a strategy for treating the disease. It is therefore important to identify all members of the genetic pathway in which a particular gene functions. Model organisms that can be genetically manipulated are particularly useful for such studies, as exemplified by experiments indicating that receptor and signaling proteins involved in metabolism, development and longevity in *C. elegans* are homologous to components of the insulin and transforming growth factor-β (TGF-β) pathway in humans. Recent investigations have revealed that the downstream targets of the *C. elegans* pathways are members of the forkhead transcription factor family, suggesting that human orthologs of these factors may be critical in insulin signaling[17,18]. The genes encoding key human forkhead transcription factors may be aberrantly regulated in diabetes mellitus and may present novel targets for the development of antidiabetes drugs.

As the early developmental stages of model organisms such as the frog and chick are easily accessible, it is possible to examine the expression of candidate target genes during vertebrate embryogenesis to obtain possible clues as to function. The number of known genes that have an embryonic function and are later associated with disease, or might be therapeutic in adults, is increasing, and expression analyses can often suggest functional activity in the adult.

A biotechnology industry is emerging to take advantage of this developmental biology based approach to functional genomics. For example, the relatively new companies of Ontogeny, Exelixis, Nemapharm (a division of Sequana Therapeutics) and Cadus are using a variety of model organism approaches to probe gene function. In addition, companies such Lexicon Genetics are focusing on the production of knockout mice for genes in the mouse genome.

Apart from using model organisms for gene functional studies, some biotech companies are using model organisms such as *C. elegans* and yeast directly for drug discovery and lead identification. For example, mutants of *C. elegans* can be grown and analyzed in high-throughput mode in screening studies to identify small molecules that may rescue a particular mutant phenotype. In this case it is assumed that the similarity between worm and human proteins is such that leads derived from the worm screen will have activity against the homologous human protein. This assumes similarity not only in gene function and genetic pathways but also in the three-dimensional structure of the gene products; it remains unproven as a method for lead identification. In other approaches, yeast strains can be engineered to express human genes such as receptors, and can be used in screening strategies to identify novel or surrogate receptor ligands.

Embryonic proteins as drugs or drug targets

Many proteins that are known to be important regulators of embryonic development, especially signaling proteins and growth factors, may have application as therapies to treat human disease. As drugs, such proteins would stimulate specific developmental processes, resulting in the replacement of specialized cell types that have been lost through disease or injury. These approaches require that appropriate receptors and signaling pathways remain intact in the adult target tissues. Examples of the applications and current status of some of these factors are given in Table 2. Several proteins are already widely used as drugs. For example, recombinant human erythropoietin (Epogen, from Amgen) is used to treat patients with chronic kidney disease who develop anemia from lack of erythrocytes. Erythropoietin, a protein normally produced by the kidney, stimulates progenitor cells in the bone marrow to form mature erythrocytes. A second Amgen product, Neupogen, is a recombinant form of granulocyte colony-stimulating factor and is used to treat neutropenia caused by chemotherapy or marrow transplantation.

Growth and signaling factors

Basic fibroblast growth factor (bFGF) has neuroprotective and angiogenic (blood vessel forming) properties. Scios is developing recombinant forms of bFGF for the treatment of stroke, coronary artery disease and peripheral vascular disease. Vascular endothelial growth factor (VEGF), another protein that promotes angiogenesis, is being tested by Genentech as a potential treatment for coronary artery disease. By contrast, specific inhibitors of growth factors such as VEGF may be useful in counteracting neovascularization associated with diseases such as diabetic retinopathy[19].

TGF-β and members of the large superfamily of TGF-β-related proteins are under extensive investigation as potential

Table 2. Examples of embryonic proteins under consideration for use as therapeutic agents or which may be targets of therapies[a]

Protein	Application	Status	Company
Erythropoietin (*Epogen*)	Chronic renal failure	On the market	Amgen
Granulocyte colony stimulating factor (G-CSF) (*Neupogen*)	Chemotherapy or marrow transplant-induced neutropenia	On the market	Amgen
Basic fibroblast gowth factor (bFGF) (*FIBLAST*)	Coronary heart disease Peripheral vascular disease Stroke	 Phase II/III clinical trials	Scios/Wyeth-Ayerst
Osteogenic protein-1 (OP-1/BMP-7)	Bone fractures	Phase III clinical trials complete	Creative BioMolecules/ Stryker
	Renal disease	Preclinical trials	Biogen/Creative BioMolecules
Nerve growth factor (NGF)	Peripheral neuropathies	Phase III clinical trials	Genentech/CytoTherapeutics
Brain-derived neurotrophic factor (BDNF)	Amyotrophic lateral sclerosis (ALS)	Phase II/III clinical trials	Amgen/Regeneron
Ciliary neurotrophic factor (CNTF)	Neural degenerative diseases Amyotrophic lateral sclerosis	Phase II clinical trials	CytoTherapeutics
Glial cell line-derived neurotrophic factor (GDNF)	Parkinson's disease	Phase I/II clinical trials	Amgen
Neurturin (GDNF)		Phase I clinical trials	Genentech/CytoTherapeutics
Keratinocyte growth factor (KGF)	Oral and gastrointestinal mucositis	Phase I/II clinical trials	Amgen
Vascular endothelial growth factor (VEGF)	Coronary arterial disease	Phase I clinical trials	Genentech
Bone morphogenic protein-2 (BMP-2)	Repair and augmentation of bone and cartilage	Phase I clinical trials	Genetics Institute
Growth and differentiation factor 8 (GDF-8)	Muscle-wasting	Research	MetaMorphix
Growth and differentiation factor 9 (GDF-9)	Fertility	Research	MetaMorphix
Hedgehog proteins	Alzheimer's disease; fracture repair; implant fixation; osteoarthritis; osteoporosis; Parkinson's disease	Research	Ontogeny/Biogen Ontogeny/Boehringer Mannheim

[a]The status of testing of a particular drug changes frequently; the data given here are meant to convey the diversity of potential protein therapeutics at various stages of testing and approval

drugs to treat cancers and other diseases[20]. This group of proteins includes the bone morphogenetic proteins (BMPs) and the growth/differentiation factors (GDFs). BMPs have diverse effects during development, including regulation of cell differentiation, proliferation, apoptosis and morphogenesis[21,22]. They are involved in the development of the nervous system, lungs, kidneys, gonads, skin and somite-derived structures such as muscle and the skeleton. BMPs that are under investigation as agents for the repair and augmentation of bone and cartilage include osteogenic protein-1 (OP-1/BMP-7), produced by Creative BioMolecules/Stryker, and BMP-2, produced by Genetics Institute. OP-1 is also being developed as a therapeutic agent for acute and chronic kidney failure by Creative BioMolecules/Biogen. The GDFs are also important regulators of tissue growth and development. GDF-8 is specifically expressed in skeletal muscle, and mice with disrupted *GDF-8* genes have significantly increased muscle mass, suggesting that GDF-8 is a negative regulator of skeletal muscle growth[23]. MetaMorphix is investigating whether antagonists of GDF-8 action would be useful in the treatment of muscle wasting. GDF-9 is an oocyte-derived growth factor that is required for early ovarian folliculogenesis[24], and may be a useful agent in the treatment of fertility diseases.

The *hedgehog* gene was originally identified as encoding a regulator of segmentation during *Drosophila* embryonic development by the genetic screens of Nüsslein-Volhard

and Wieschaus[25]. Human tissues express three forms of this inducing protein: Desert hedgehog, Indian hedgehog, and Sonic hedgehog[26]. Recent studies indicate that Desert hedgehog is essential for development of the male reproductive system, whereas Indian hedgehog and Sonic hedgehog appear to be required for development of the vertebrate skeleton and CNS, respectively. Ontogeny is exploring the use of hedgehog proteins as therapies to treat neurodegenerative diseases such as Parkinson's and Alzheimer's, and also bone and cartilage disorders such as fracture repair, implant fixation, osteoarthritis and osteoporosis. Mutations of the *patched* and *smoothened* genes, which encode the hedgehog receptor and a target of the hedgehog signaling pathway, respectively, are associated with basal-cell carcinomas, the most common human cancer[27,28]. Future therapies could therefore also target these proteins.

Other proteins under test for the treatment of neurodegenerative diseases include glial cell line-derived neurotrophic factor, ciliary neurotrophic factor, and brain-derived neurotrophic factor for the treatment of Parkinson's disease, Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis (Lou Gehrig's disease). Nerve growth factor is being developed by Genentech/Cytotherapeutics to treat peripheral neuropathies – the progressive nerve fiber damage often occurring in the hands and feet of diabetic patients. Cytotherapeutics are exploring the use of encapsulated genetically modified cells as vehicles for the delivery of ciliary neurotrophic factor[29,30].

Nuclear proteins

Distinct types of therapy may involve the use of transcription factors – proteins that regulate gene activity. Most transcription factors are broadly expressed, but mutations of the genes encoding certain tissue-specific transcription factors have revealed that these factors are essential for the development of specific cell types or whole organ systems. For example, animals lacking the *IPF-1* gene do not develop a pancreas[31], mutation of the gene encoding SF-1 results in animals without gonads and adrenals[32], and *Pit-1* gene defects cause specific loss of pituitary cell types and dwarfism[33]. Ontogeny is investigating IPF-1 as a potentially useful agent or target in therapies for diabetes. Others are exploring the use of ligands that activate or modulate the activity of the transcription factors that regulate specific developmental pathways. For example, peroxisome proliferator activated receptor-γ (PPARγ) is a nuclear receptor transcription factor that, in combination with members of the CAAT/enhancer-binding proteins, regulates adipocyte differentiation in mammals[34]. PPARγ activity may also be critical in lipid metabolism and insulin sensitivity, and the PPARγ gene is therefore a candidate gene for human disorders such as obesity and type 2 diabetes mellitus. The endogenous ligands for PPARγ appear to be prostaglandin derivatives, and synthetic antidiabetes thiazolidinedione drugs may also work by binding to PPARγ and modulating its activity[35].

Embryonic cells as therapeutic agents

Embryos and adult organisms contain populations of undifferentiated cells, known as stem cells, that serve as a source of cells for populations of one or more types of differentiated cell lineages. Stem cells are capable of self-renewal – they can create more stem cells as well as providing differentiated progeny. In adults, some tissues and organs contain stem cells that serve as a reserve population of cells and that respond, at varying rates, to accidents or changing environments by providing cells that can undergo further development within the organism. Transplanted stem cells are potentially powerful therapeutic agents for the treatment of many human diseases. They have been proposed as therapies for wound repair, neurological diseases, heart disease, liver diseases, blood/immune diseases and many other disorders[36,37].

In such applications, transplanted stem cells replace failed cells and develop into functional tissue. Further, the transplanted cells may release important molecules locally (in physiological quantities and forms), thereby facilitating the function of existing cells and tissues. Transplanted stem cells are also likely to be appropriately regulated by physiological signals within the patient, responding at precise sites and times. Other advantages of this approach might be that applications only require a one-time treatment and that, if the stem cells are provided in an encapsulated form, the transplanted cells could be confined to a specific area and retrieved later if required. Possible drawbacks to the use of transplanted stem cells include immunorejection of the cells and difficulty in controlling the differentiation of the cells to specific terminal phenotypes.

A powerful use of therapeutic strategies that are based on developmental biology might involve the combined use of embryonic stem cells and developmentally important

embryonic proteins. In these approaches, embryonic stem cells engineered to express specific developmental regulatory proteins could serve as self-renewing vehicles for gene therapies targeted to specific tissues, or the expressed proteins could enhance the potential of the stem cells to initiate or facilitate repair. In addition to their potential use as direct therapies and as vehicles for gene therapy, native and genetically engineered embryonic primordial stem cells may also be useful tools in drug screening strategies, including the testing of embryonic proteins as therapeutic agents.

Sources of transplanted cells

Sources of transplanted cells for the treatment of human disease include autologous/autogeneic cells (from the same individual), allogeneic cells (from other individuals) and xenogeneic cells (from different animal species). Cells from each source have advantages and disadvantages. Autogeneic cells are a perfect genetic match, and therefore do not require strategies designed to avoid immunorejection of the cells. However, autogeneic cells are likely to be in limited supply, could be difficult to purify and might be diseased. Allogeneic cells might also be in limited supply and would probably require the use of immunosuppressive drugs to avoid rejection. Ethical concerns could limit or prohibit the use of human embryonic or fetal cells and tissues. The development of human or primate embryonic stem cell lines might provide a renewable source of pluripotent cells for transplantation and help circumvent some ethical concerns. Xenogeneic cells might be more easily obtainable – from primate or pig donor animals, for example – but, again, would require the use of strategies or therapies to avoid rejection of the cells. Encapsulation of transplanted cells in devices that allow molecules to diffuse to and from the cells, but which prevent the entry of complement components and lymphocytes, could reduce or eliminate the need for immunosuppression.

Alternative strategies to avoid rejection of transplanted cells without general immunosuppression of the patient are being explored by companies such as Alexion. These strategies involve the use of donor cells from transgenic animals that express human immunoregulatory molecules that inhibit or deceive the recipient rejection process[36–38]. The use of donor animals with relatively large litter sizes, such as pigs, has generated much interest. Several studies have suggested, however, that even donor animals such as pigs, as well as human and primate donors, represent potential sources of infection with retroviruses[39]. Donor cells might therefore have to be screened before transplantation to avoid transmitting such viruses. Recent advances in cloning large animals such as sheep[40] could make optimized, homogeneous sources of organs for xenotransplantation readily available in the future.

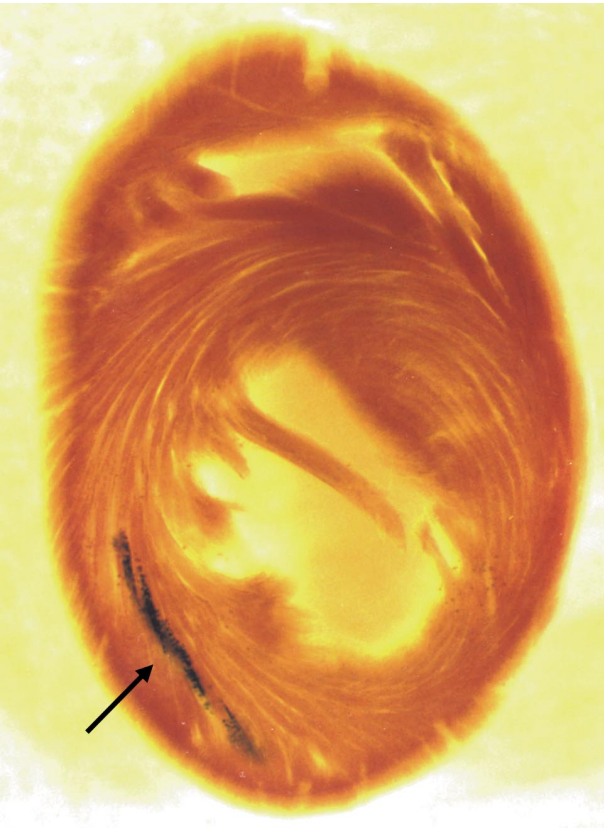

Figure 1. *Coronal section through the heart of an adult mouse that was the recipient of a graft of fetal cardiomyocytes from donor transgenic mice expressing nuclear β-galactosidase. Grafted cells convert X-gal substrate to a blue precipitate (arrow) and become functionally incorporated into the recipient cardiac tissue (see Ref. 48). Photograph courtesy of Dr Loren Field, Indiana University.*

Cells from the nervous system

Stem cells are being explored as therapies for multiple diseases of the nervous system, including neurodegenerative diseases (Huntington's, Parkinson's, Alzheimer's), amyotrophic lateral sclerosis, retinal degeneration, brain tumors, and myelination disorders[41–45]. The brain is an immunoprivileged site, and implantation of embryonic neural stem

cells to treat diseases of the brain might not demand the use of the immunosuppressive strategies that are required for treatments involving other organs.

Remyelination has been observed following allogeneic and xenogeneic transplantation of oligodendrocyte/astrocyte precursor cells into chemically or impact-induced demyelinated lesions of the rat spinal cord[46,47]. In addition, dopaminergic cells from fetal human cells have been used to treat Parkinson's disease[48,49], and similar cells obtained from fetal pigs (Neurocell-PD, Diacrin/Genzyme) are in Phase I trials. γ-Aminobutyric acid releasing fetal pig brain cells are also being examined in Phase I trials for the treatment of Huntington's disease (Neurocell-HD, Diacrin/Genzyme). Other potential applications of embryonic neural cells include the use of cholinergic cells, obtained from fetal adrenal medulla for example, as therapy in the treatment of Alzheimer's disease and as analgesics.

Cardiac cells

Recent experimental approaches have suggested that fetal cardiac cells may be used to replace damaged cardiac muscle in the treatment of heart disease. Field and coworkers have elegantly shown that transplanted mouse or dog fetal cardiomyocytes can functionally populate the ventricular myocardium of recipient adult mice or dogs[50,51]. Cardiac cells from transgenic donor animals expressing β-galactosidase can readily be identified and studied in the hearts of recipient animals (Fig. 1). Further, these researchers have generated pure populations of cardiomyocytes from *in vitro* cultures of differentiated pluripotent embryonic stem cells and demonstrated that these cells can also be effectively grafted and incorporated into adult heart muscle[52]. These studies should guide future protocols for the treatment of myocardiopathies and myocardial infarction.

Hematopoietic stem cells

Hematopoietic stem cells (HSCs) are cells that have the capacity to give rise to all the cells of the hematopoietic system. They are found first in the yolk sac of the developing embryo, then later in the fetal liver and bone marrow. HSCs derived from the yolk sac, fetal liver, umbilical cord blood, peripheral blood and bone marrow are all candidates for use as therapies to treat hematopoietic diseases, (auto)immune disorders and graft-versus-host disease. Allogeneic bone marrow transplants are a relatively common and powerful therapy, but often fail because of immunorejection and graft-versus-host disease[53]. Broxmeyer and coworkers have demonstrated the exciting potential of autologous or closely matched umbilical cord blood as a source of HSCs in the treatment of a variety of blood disorders, including anemias and leukemias[53]. Systemix are investigating the use of yolk sac stem cells (YS-HSCs). YS-HSCs can be expanded *in vitro*, and transplanted YS-HSCs may be less immunoreactive than cord, bone or liver derived HSCs because they lack expression of major histocompatibility complex associated antigens[54]. Like the other types of stem cells considered in this review, HSCs could present valuable targets and vehicles for gene therapies of blood/immune diseases[53].

Mesenchymal stem cells

In addition to hematopoietic precursor cells, the adult bone marrow contains mesenchymal stem cells (or marrow stromal cells, MSCs). MSCs can differentiate to form bone, cartilage, skeletal muscle, and adipocytes. The normal function of these cells is unclear, but they have potential for use – either alone, or after modification so that they express regulatory proteins such as bone morphogenetic proteins – in the treatment of soft tissue wounds, and diseases of tendon, cartilage and bone, including osteoporosis[55,56]. For example, companies such as Osiris and MorphoGen are investigating the use of autologous MSCs, that have been extracted from the bone marrow or other tissues of patients and expanded in culture, to repair joint surfaces in the treatment of arthritis or to restore damaged bone. A further use of MSCs might be as effective vehicles for gene therapy whereby MSCs are engineered to express a therapeutic protein and then returned to the patient to repopulate the bone marrow and secrete the protein.

Applications in animal agriculture

Increasing the efficiency of animal protein (milk and meat) production is highly desirable in the animal agricultural industry. For most agricultural animal species the time spent *in ovo* or *in utero* is a large percentage of the life of the animal to market (Table 3), but until recently this early part of the animal's lifetime has been overlooked, and attempts to increase productivity have focused on the posthatch/postnatal phase of animal growth. The possibility of influencing the embryonic development of livestock species with drugs so as to achieve permanent,

Table 3. *In ovo*/gestation times for various agricultural species

Animal	Average time from birth to market	Gestation period	Gestation as % of growth period
Broiler chickens	42 days	21 days	33%
Beef cattle	20 months	9 months	31%
Swine	190 days	114 days	37%
Sheep	5.5–6 months	5 months	83–91%

positive effects on posthatch/postnatal growth and development has only recently been experimentally addressed. This application of developmental biology to drug discovery, where the embryo itself is the direct target of drug delivery, is unique to the animal health industry and has a potential that is only just beginning to be realized. The field is still in its infancy, however; no drug has yet been approved that works in this manner and only time will tell if this approach lives up to its initial promise.

There are several advantages to a developmental biology based experimental approach to the enhancement of animal productivity. First, it is possible to effect changes that are impossible at other times; for example, influences on muscle mass through changes in muscle fiber number can only be achieved during a particular window of sensitivity in early development (see below). Because these approaches target a particular differentiation event during development, the time when the drug needs to be delivered is often narrow, suggesting that in some cases a single dosage would be sufficient to achieve the desired effect. Further, since the drugs are influencing a dynamic period of differentiative events, the effect would in many cases be expected to be irreversible. In addition, as the drug is delivered so early in the life of the animal and often for a limited period, the period of time between termination of drug delivery and slaughter is near the maximum and of the order of weeks. This would be expected to minimize the risk of drug residues in the meat derived from the treated animal – a concern for animal health pharmaceuticals. An added benefit of this approach is that, for those mammalian species with multiple births, such as the pig, it is possible to affect multiple offspring by treating one animal, the mother, during pregnancy.

Mechanisms for drug delivery to embryos and fetuses of agricultural animals are already available. Over 75% of broiler chicken eggs are injected *in ovo* by an automatic injection device present in most chicken hatcheries[57]. The machine is currently used to deliver vaccines and antibiotics to chickens prior to hatching, but the technology could be adapted to early embryonic delivery, and certainly the notion of *in ovo* drug delivery is already accepted by the poultry industry. For mammals (swine, cattle, sheep), the delivery would be indirect through the mother, via injections, implants or as feed additives.

Several embryonic events are potentially subject to manipulation – the consequences of which would be of interest to animal agriculture. These events include growth, sexual differentiation, fat development, muscle development and development of the immune system. The following three examples in the area of muscle development and sexual differentiation illustrate the current status of this approach to drug discovery. Increasing the muscle mass of meat-producing animals can be achieved in two ways: by increasing muscle fiber size (hypertrophy) or muscle fiber number (hyperplasia). Increasing muscle mass through an increase in muscle cellularity has been stated as being the most important parameter with regards to increased muscle growth and quality[58], and there is documented evidence of a positive correlation between muscle fiber number and increased growth efficiency in the pig[59]. Muscle fiber number is determined during embryonic development; for example, in the pig, muscle fiber number reaches a maximum by day 70 of gestation, with birth occurring at days 110–116 (Ref. 60). In order to influence muscle mass through muscle fiber number, drug delivery *in ovo* or *in utero* is essential. Daily delivery of porcine somatotropin (growth hormone) by injection to gestating swine during a sensitive window of gestation (days 10–45) has been shown to lead to an increase in muscle fiber number of the piglets at birth and subsequently to improved carcass characteristics of these offspring at market weight[61,62].

Kocamis and coworkers[63] have attempted to manipulate the growth of chickens by administering insulin-like growth factor-I (IGF-I) to developing embryos *in ovo*. Injection of IGF-I during the first week of egg incubation has been reported to significantly increase skeletal muscle growth of broiler chicks. During posthatch, chicks that had accelerated and greater muscle growth demonstrated better feed efficiency.

Male chickens are preferred in the broiler industry because males grow more efficiently than females. The possibility of influencing the sexual differentiation of

genetically female chickens so that they phenotypically resemble males, including male growth patterns, has been considered. In the first week of incubation *in ovo*, the chicken gonadal system goes through an 'indifferent' period during which it can develop as testes or ovaries and is greatly influenced by the levels of circulating steroid hormones. Injection of inhibitors of aromatase (the enzyme that converts testosterone to estradiol) on day 5 of egg incubation leads to sex reversal of genetic females; the treated birds, although sterile, look outwardly male, develop testes and undergo spermatogenesis[64].

Conclusion

Current research in developmental biology will provide many of the tools and discoveries required to identify and develop a new generation of biological drugs. These drugs may be used in the treatment of many major diseases and conditions, including heart disease, diabetes, cancer, neurodegenerative disorders, blood/immune diseases, kidney dysfunction, arthritis, osteoporosis and infertility. In addition, direct manipulation of the embryo may enable significant improvements in productivity and quality for the animal agriculture industry.

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