RNA INTERFERENCE AS A TOOL FOR CHICKEN FUNCTIONAL GENOMICS

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SUMMARY
Functional analysis of newly identified genes has become a major part of genomic projects and will obviously become important for gene discovery and annotation of the recently sequenced chicken genome. Mutagenesis and transgenic approaches have become routine for functional genomic studies in species such as mice (Okabe et al., 1997), zebrafish (Gong et al., 2002) and drosophila (Link, 2002), however these techniques are still being developed for chickens and to date remain far from routine. A recent phenomenon known as RNA interference (RNAi) promises to advance the elucidation of gene function in chicken cells. We have evaluated and demonstrated the knockdown potential of both siRNA and shRNA molecules in the chicken DF1 cell line.

Keywords: RNAi, gene silencing, functional genomics

INTRODUCTION
RNAi is a method of sequence specific gene knockdown and has become an incredibly useful tool to analyse gene function in both plants and animals (Napoli et al., 1990; Sharp, 1999; Elbashir et al., 2001). RNAi describes the post-transcriptional silencing of gene expression in response to the introduction of double-stranded RNA (dsRNA) into cells. The conserved RNAi pathway involves the processing of dsRNA duplexes into 21-23 nucleotide (nt) molecules known as small interfering RNAs (siRNA) to initiate gene knockdown (Fire, 1999; Bosher and Labouesse, 2000; Hannon, 2002). Since the discovery of RNAi in animals (Fire et al., 1999) the use of long dsRNA in lower eukaryotes, especially in the model organism Caenorhabditis elegans, has been used to determine gene function (Barstead, 2001; Ashrafi et al., 2003). However, in vertebrate systems the cellular uptake of long dsRNA induces an antiviral defence mechanism initiated by interferon (IFN), leading to non-specific translational shutdown and apoptosis (Williams, 1997; Stark et al., 1998; Gil and Esteban, 2000). This non-specific cellular activity can be circumvented by the direct transfection of either chemically synthesised or in vitro transcribed siRNAs of approximately 21 nt in length into mammalian cells (Tuschl, 2002). These short molecules do not activate the IFN response, but can induce reliable and efficient transient knockdown of target genes (Dykxhoorn et al., 2003; Duxbury and Whang, 2004). As a consequence, the development of DNA-based vectors for expression of short hairpin RNA (shRNA) molecules that are processed within the cell to produce active siRNA molecules have been developed (Brummelkamp et al., 2002; Yu et al., 2002; Paddison et al., 2002). Such DNA expression constructs have achieved highly efficient gene knockdown without induction of the IFN response.

There are very few reports of the use of RNAi for gene function analysis in chicken cells, therefore we have evaluated and demonstrated the knockdown potential of both siRNA and shRNA molecules in the chicken DF1 cell line. We used a plasmid (pEGFP–N1, Clontech) encoding enhanced green fluorescent protein (EGFP) as the target gene for RNAi.
MATERIALS AND METHODS
The target sequence for our EGFP siRNA and shRNA molecules has been reported previously (Kim and Rossi, 2003). Scrambled siRNA and shRNA sequence has also previously been reported (Kim and Rossi, 2003). siRNAs were synthesized using the Ambion Silencer siRNA construction kit and shRNAs were expressed from the Ambion pSilencer 1.0 plasmid. The chicken DF1 cell line (P142 chicken embryonic fibroblast ATCC CRL-12203) was cultured in Dulbecco’s modified eagle’s medium (DMEM) containing 10% fetal calf serum, 2 mM glutamine, 10 mM HEPES, supplemented with penicillin (100 U/ml) and streptomycin (100 µg/ml) and incubated in humidified atmosphere containing 5% CO₂ at 37°C. DF1’s were co-transfected with pEGFP-N1 (500 ng) and siRNAs (100 nM, 50 nM, 10 nM, 1 nM) or shRNA vector (500 ng) using Lipofectamine 2000 (Invitrogen) and transfected cells were dispensed into 8-well chamber slides. EGFP expression was monitored at 72-hour post-transfection using fluorescence microscopy (Leica DMLB).

RESULTS AND DISCUSSION
Both siRNAs (50 nM and 100 nM) and pSilencer 1.0 expressed shRNAs triggered dramatic silencing in excess of 90% of EGFP gene expression in DF1 cells (Figure 1). Therefore, RNAi gene silencing by means of introducing siRNA or shRNA molecules into cultured chicken cells shows great promise for in vitro functional genomic studies.

Application of RNAi in experimental systems for both human and mouse has already provided a great leap forward in the elucidation of gene function. Genome wide siRNA libraries exist for both species and specialized siRNA transfection techniques have been developed for numerous cell lines of both species. To facilitate large-scale functional genomics studies using RNAi, several high throughput approaches have been developed based on microarray or microwell assays (Ziauddin and Sabatini 2001). Recent establishment of the large genome wide libraries of RNAi reagents combined with a variety of detection assays has for the first time opened the door for genome-wide screens of gene function in mammalian cells and will no doubt extend into similar screens in chicken cells.

Chicken embryos have been widely used as a model system for the investigation of vertebrate embryogenesis (Eguchi and Okada, 1973), because they can be easily manipulated compared with other vertebrates. Sato et al. 2004 have recently silenced EGFP gene expression in the early stages of development of a chick embryo by electroporation of siRNAs. The development of RNAi in chicken embryos could lead to a powerful tool for use in the functional analyses of genes expressed during embryogenesis and will help path the way for in vivo functional genomic studies.
Figure 1. RNAi silencing of EGFP gene expression in cultured DF1 cells.
A. DF1 cells co-transfected with pEGFP-N1 and siRNAs at 100 nM, 50 nM, 10 nM and 1 nM.
B. DF1 cells co-transfected with pEGFP-N1 and pSilencer 1.0 vector expressing shRNAs. EGFP expression was monitored at 72-hour post-transfection using fluorescence microscopy. The control scrambled siRNA and shRNA sequence has previously been reported (Kim and Rossi, 2003).

REFERENCES
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