Supporting Materials and Methods.

Differential detergent fractionation (DDF) isolation of oocyte and cumulus cell proteins

Predominantly cytosolic proteins were extracted first by six sequential 10 min incubations in a buffer containing digitonin; next a fraction predominantly containing membrane proteins was isolated by incubating the cells in 10% Triton X-100 buffer for 30 min and removing the solubilized proteins. A deoxycholate buffer is then added to the remaining insoluble material and subjected to freeze-thawing to disrupt the nucleus. The sample was then aspirated through an 18 gauge needle and treated (37°C, 1h) with a mixture of DNase I (50 U; Invitrogen, Carlsbad CA), and RNase A (50 mg; Sigma-Aldrich, St Louis, MO) to digest intact nucleic acids. Predominantly nuclear proteins are collected from the resulting soluble fraction. Any remaining pellet, containing the least soluble proteins, is treated with a buffer containing 5% SDS.

Proteomics

Proteins were precipitated with 25% trichloroacetic acid to remove salts and detergents. Protein pellets were resuspended in 0.1 M ammonium bicarbonate, 5% HPLC grade acetonitrile (ACN), reduced (5mM DTT, 65 C, 5 min), alkylated (10 mM iodoacetamide, 30°C, 30 min) and then trypsin digested until there was no visible pellet (1:50 w/w 37°C, 16 h). Peptides were desalted using a peptide microtrap (Michrom BioResources, Inc., Auburn, CA) and eluted using a 0.1% trifluoroacetic acid, 95% ACN solution. Desalted peptides were dried in a vacuum centrifuge and resuspended in 20μL of 0.1% formic acid. LC analysis was accomplished by strong cation exchange (SCX) followed by reverse phase (RP) LC coupled directly in line with ESI ion trap mass spectrometer (LCQ Deca XP Plus; ThermoElectron Corp, San Jose, CA). Samples were loaded into a LC gradient ion exchange system (Thermo Separations P4000 quaternary gradient pump coupled with a 0.32 × 100 mm BioBasic strong cation exchange column). A flow rate of 3μL/min was used for both SCX and RP columns. A salt gradient was applied in steps of 0, 5, 10, 15, 20, 25, 30, 35, 40, 45,50, 57, 64, 71, 79, 90, 110, 300, and 700 mM ammonium acetate in 5% ACN, 0.1% formic acid and the resultant peptides loaded directly into the sample loop of a 0.18 × 100 mm BioBasic C18 RPLC column (ThermoElectron). The reverse phase gradient used 0.1% formic acid in ACN and increased the ACN concentration in a linear gradient from 5% to 30% in 30 min and then 30% to 65% in 9 min followed by 95% for 5 min and 5% for 15 min. The spectrum collection time was 59 min for every strong cation exchange step. The mass spectrometer was configured to optimize the duty cycle length with the quality of data acquired by alternating between a single full MS scan followed by three tandem MS scans on the three most intense precursor masses (as determined by Xcalibur software in real time) from the full scan. The collision energy was normalized to 35%. Dynamic mass
exclusion windows were set at 2 min, and all of the spectra were measured with an overall mass/charge (m/z) ratio range of 300-1700.

Mass spectra and tandem mass spectra were used to search subsets of the nonredundant protein database (NRPD) downloaded from the National Center for Biotechnology Institute (NCBI; 7/20/05) using TurboSEQUEST (Bioworks Browser 3.2; ThermoElectron). We used a bovine subset (BovDb; search terms: Bos AND taurus) of the NRPD, which gave 39,963 entries and included "PREDICTED" and "Hypothetical" proteins. Trypsin digestion, including mass changes due to cysteine carboxamidomethylation and methionine oxidation, was applied in silico to each database. The peptide (MS precursor ion) mass tolerance was set to 1.5 Da, and the fragment ion (MS²) mass tolerance was set to 1.0 Da. Peptide matches were included if they were ≥6 amino acids long and had ∆Cn > 0.1 and Sequest cross correlation (Xcorr) scores for charge states of 1.9, 2.2 and 3.75 for +1, +2, and +3 respectively (Washburn et al., 2001). All protein identifications and their associated MS data have been submitted to the PRoteomics IDEntifications database (PRIDE; Martens et al., 2005).

The NRPD contains all known protein sequences, and so it is redundant at the level of protein identity, i.e. the “non-redundancy” of protein databases is at the amino acid sequence level, not at the protein identity level. Because tandem MS often does not distinguish subtle sequence variants our initial protein lists contained protein identification redundancies. We manually identified and removed these redundancies from our results.

**Gene Ontology Annotation**

Gene Ontology (GO) has become a standard method for gene product functional annotation used for systems biology and other functional genomics data modeling (McCarthy et al., 2005). GO describes gene products in terms of cellular component, biological process and molecular function. We first GO-annotated all proteins in our datasets using existing annotations from probable orthologs with ≥90% sequence identity using the UniRef 90 database. UniRef90 is a clustered set of sequences from the UniProt KB (including splice variants and isoforms) and selected UniParc records clustered such that each cluster is composed of sequences that have at least 90% sequence identity. Proteins with between 70-90% sequence identities were annotated from presumptive orthologs using the GOanna tool at AgBase (McCarthy et al., 2006a). GOanna uses a BLAST strategy to search entries in the UniProtKB and the results were manually inspected based on alignment identity and features information (i.e. functional domains, motifs, and active sites). All proteins annotated in to either Uniprot90 or using GOanna carry the GO evidence code “inferred from sequence similarity” (ISS).