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A Mouse Model of Diet-Induced Obesity and Insulin Resistance

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Abstract

Obesity is reaching pandemic proportions in Western society. It has resulted in increasing health care burden and decreasing life expectancy. Obesity is a complex, chronic disease, involving decades of pathophysiological changes and adaptation. Therefore, it is difficult ascertain the exact mechanisms for this long-term process in humans. To circumvent some of these issues, several surrogate models are available, including murine genetic loss-of-function mutations, transgenic gain-of-function mutations, polygenic models, and different environmental exposure models. The mouse model of diet-induced obesity has become one of the most important tools for understanding the interplay of high-fat Western diets and the development of obesity. The diet-induced obesity model closely mimics the increasingly availability of the high-fat/high-density foods in modern society over the past two decades, which are main contributors to the obesity trend in human. This model has lead to many discoveries of the important signalings in obesity, such as Akt and mTOR. The chapter describes protocols for diet induced-obesity model in mice and protocols for measuring insulin resistance and sensitivity.

Keywords

Obesity; High-fat diet; Insulin resistance; Body weight; Metabolism

1. Introduction

Obesity results from an imbalance of food intake, basal metabolism, and energy expenditure. At an individual level, multiple endogenous or environmental causes could lead to obesity (1). However, in most cases, a combination of excessive caloric intake and availability of energy-dense meals is thought to be the main contributor to obesity (2). Because complications from obesity such as diabetes and cardiovascular disease usually require decades, surrogate animal models are important for studying the molecular aspects of obesity and its pathophysiological effects. One of these models that are gaining increasing attention is the diet-induced obesity model in mouse.

Foods that are rich in fats have been shown to produce increased body weight and diabetes in various strains of mice and rats (3). In the past 20–30 years, there have been many studies characterizing the responses of animals exposed to high-fat diets (4). Some animals show profound increases in their body fat content while some are resistant to weight gain with high-fat diet (5). For example, the outbred Spraque-Dawley rats when fed with high-fat diet have different responses with regard to the development of obesity. In the mice, the A/J mouse and C57BL/KsJ mouse are relatively resistant to high-fat diet when compared to C57BL/6J mouse (6). The B6 mouse is a particularly good model mimicking human metabolic derangements that are observed in obesity because when fed ad libitum with a high-fat diet, these mice develop obesity, hyperinsulinemia, hyperglycemia, and

hypertension, but when fed ad libitum to chow diet, they remain lean without metabolic abnormalities (8).

The metabolic abnormalities of B6 mouse closely parallel that of human obesity progression pattern. Although an increase in body weight can be noticed after 2 weeks, the increase is gradual and becomes apparent after 4 weeks. After 16–20 weeks of high-fat diet feeding, mouse will typically exhibit 20–30% increase in body weight when compared to chow-fed mouse (9). The high-fat diet's effects on blood glucose are more discrepant and depend on the type of dietary regimen. Hyperglycemia usually develops within 4 weeks of a high-fat diet (11). The elevation of fasting glucose is usually accompanied by increases in fasting insulin levels. At that time, hyperinsulinemic-euglycemic clamp experiments will demonstrate whole-body insulin resistance. However, there are no reliable predictors for diabetes development or onset and the reported development of overt diabetes is controversial. Nevertheless, the full manifested picture of obesity develops after 16 weeks of high-fat diet with adipocyte hyperplasia, fat deposition in mesentery, increased fat mass, diabetes, and hypertension (12).

Akt and mTOR pathyway integrates several important signals that regulate cell growth and metabolism. Activation of Akt and mTOR signalings by food intake has great implications in obesity and insulin resistance (9). Inhibition of Akt and mTOR pathway by rapamycin has effects in longevity (13), adipocyte differentiation, and obesity (14). Recent studies have shown that S6K1-deficient mice and Akt1 knockout mice exhibit are prevented from diet-induced obesity through model of murine high-fat diet induced obesity described below (15).

Several factors are also important when using this model of diet-induced obesity. First, the genetic nature and degree of model characterization as well as differences in mouse background could lead to different phenotype (16). For examples, AKR/J and DBA/2J mice are very responsive to high-fat diet, while A/J and Balb/cJ mice are more resistant. C57BL/6J mouse are better characterized than some of the other models. Second, gender usually plays an important role. Generally, male mice are more affected by diabetes than are female mice and thus are used more often in diet-induced obesity studies (17). Third, environmental factors are important and should be considered. Obese mice are sensitive to stress and their environmental settings will affect experimental results (18). Therefore, cage placement, mice density, food quality, mice handling, beddings and mice-check frequency will all results in disturbance of the development of obesity in experimental mice.

2. Materials

2.1. Mice

1. C57BL/6J males, 4–6 weeks old (The Jackson Laboratory, stock 000664).
2. Inventoried (DIO) C57BL/6J males (The Jackson Laboratory) (see Note 1).

2.2. Diet

1. 10 kcal% fat diet (Standard diet) (see Note 2).

[1]Jackson Laboratory provides various diets, which could be used to induce obesity. For example, C57BL/6J mice could be fed with 60% high-fat diet starting at 6 weeks of age while control mice are fed with 10% fat diet. The age availability is from 6 to 26 weeks. They have established physiological database and inventoried mice that could save much time for experiments. Certain care should be managed when using these mice. First, effect of shipping should be taken into account as mice tend to lose weight in transit. We usually acclimate the mice for 1 week in their experimental environment before using them. Second, we usually do not mix these mice cohort with each other. Male mice tend to fight, which will have some impact on their body weight.

2. 45 kcal% fat diet (Research Diets, D12451i).
3. 60 kcal% fat diet (Research Diets, D12492i) (see Note 3).

2.3. Glucose and Insulin Test

1. Dulbecco's phosphate-buffered saline (D-PBS), 1× Liquid (Gibco 14190-136).
2. NaCl 0.9% sterilized, Baxter IV solutions (Baxter 6E1322).
3. Disposable sterile animal feeding needles, Popper & Sons, 20 G × 1 1/2 in. (VWR 20068-666).
4. Tuberculin syringe with 27 G × 1/2 in. needle (Becton- Dickinson, 309623).
5. Heated hard pad.
6. Clean cages with water bottles.
7. D-(+)-glucose 99.5% (Sigma, G7528).
8. Human insulin: Novolin R, 100 U/ml, 10 ml (NovoNordisk).
9. Scalpel.
10. Mineral oil (Sigma M3516).
11. Paper towels.
12. Ascensia ELITE XL Bayer Glucometer Blood (Bayer).
13. Ascensia ELITE XL test strips (Bayer).
14. Batteries, 3 V Lithium cell (Sigma, B0653).
15. SurePrep™ capillary tubes, 75 mm, self-sealing (Becton Dickinson 420315).
16. MicroWell 96-well polystyrene plates, round bottom (non-treated), sterile (Nunc, Sigma, P4241).
17. 20–200-μl pipette with tips.
18. Insulin (Mouse) Ultrasensitive (Alpco Diagnostics, EIA 80- INSMSU-E10).
19. Titer Plate Shaker (Thermo Scientific EW-51402-10).
20. Multilabel Readers, VICTOR (6) (PerkinElmer).

2.4. Blood Collection and Tissue Collection

1. 1-ml syringes with 25 G × 5/8 needle (Becton-Dickinson 305122).
2. Microtainer serum separator tubes (BD Diagnostics, 365956).
3. MicroWell 96-well polystyrene plates, round bottom (non-treated), sterile (Nunc, Sigma, P4241).
4. Formalin solution, neutral buffered 10% (Sigma HT501128).

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[2]Research diet D12450B. Its caloric composition is 20 kcal% protein, 70 kcal% carbohydrate and 10 kcal% fat. Every gram of its ingredient contains 3.85 kcal calorie, including 19 mg cholesterol from Lard and yellow dye.

[3]Research diet D12492. Its caloric composition is 20 kcal% protein, 20 kcal% carbohydrate, and 60 kcal% fat. Every gram of its ingredient contains 5.24 kcal calorie, including 232 mg cholesterol from lard and blue dye. It should be stored frozen and could last for 6 months. 60 kcal% fat is rarely practical when considering the human diet. However, to induce obesity in mice in shorter time and facilitate the research, 60 kcal% fat is commonly used. However, when screening the effects of drug or genetic manipulation, sometimes it may require 45 kcal% fat diet as it may be more difficult to prevent or reverse the effects of this extreme 60% kcal% diet.

5. 50-ml conical tubes (VWR 21008-178).
6. Rodent restrainer 115 VAC, animal weight range 70–125 g (Harvard apparatus).

3. Methods

Diet induced obesity model usually takes more than 16–20 weeks for completing the experiments (Fig. 1) (12). Moreover, obesity is a multiple organ system disease, involving derangements of bodyweight, adipose tissue, glucose metabolism, cholesterol level, and inflammatory markers (19). Therefore, preexperimental design is crucial for the success of the experiments. While multiple organ systems could be tested and many parameters could be examined, it is important to have upfront experimental hypothesis and goals. During obesity development, one could examine and measure a wide variety of parameters. However, different hypotheses will required different measurements and experimental methods, which in some cases, could affect physiologic parameter. These differences are detailed below.

3.1. Animal Housing

1. Keep mice under standard pathogen-free conditions with food and water ad libitum and regular 12:12 light–dark cycle. Make sure that the light–dark cycle is strictly followed, as checking mice after dark cycle started will disrupt the regular circadian rhythm of mice and will have impact on the metabolism of mice (20).
2. Mice should be housed in the same number per cages (see Note 4).
3. Check cage and bedding conditions daily but avoid check mice too frequently. Although not as frequent as other genetic modified diabetic mice, C57BL/6J mice after becoming obese and diabetic will have polyuria and mice cages may need to be changed more frequently.
4. Perform mice identification with ear notching or ear tagging at the start of the experiments, as these procedures will also affect mice body weight and glucose.
5. Pay attention to the environment nearby the mice cages. Noise, traffic, or other disturbances will affect body weight and cause bias in the diet induced obesity experiments.

3.2. Mice Randomizations and Experimental Design

As there is also individual variation even in the inbred strains of the mice, it is important to collect the baseline data of the mice before experiments. We measure body weight, tibial length, fasting glucose for mice of the same gender and age. The baseline fasting glucose of C57BL/6J male mice ranges from 40–190 mg/dl at age of 6 weeks in our laboratory; we only recruit mice with their fasting glucose between 70 and 130 mg/dl. We also exclude the male mice with body weight below 15 g or over 25 g at 6 weeks of age. Mice with ill looking, teeth problem or hair diseases are also excluded from study.

1. At age of 5 weeks, measure mouse body weight and tibial length.
2. Measure baseline glucose: insert glucometer strip into glucometer. Pick up mouse with gloves. Restrain the mice with either the other hand or the mice restrainer. We usually use unheated version of rodent restrainer. It s cylindrical and have series of

[4]As in many mammalians, C57BL/6J mice in cages also establish social hierarchies that dictate the distribution of food, water, resting places, and mating. Different mice numbers in cages will result in different social behavior and will have impact on the development of obesity.

holes on each side to facilitate injections or blood testing. Angle type restrainers are simpler if with experiences.

3. Randomize mice by body weight and exclude the mice with extreme body weight.
4. List glucose level in each experimental group.
5. Exclude mice with extreme glucose levels.
6. Perform statistic checkup to make sure that there is no grouping bias.

3.3. High-Fat Diet Feeding and Measurement of Food Intake

1. Select the different diet regimen. For example, for C57BL/6J diet induced obesity model, we usually use 60 kcal% fat diet for the experimental group and 10 kcal% chow diet for the control group, depending on the purpose of the experiments (see Note 5).
2. Measure the diet weight before change the diet. Select the diet with good particles and eliminate the fragmented food particle or powder.
3. Pour the diet into the cages and avoid pouring into the fragments or powder into the cages as they sometimes contaminated the beddings with polyuric mice. These fragments or powder will also cause error in food intake measurement.
4. Observe daily for the food color. These 60% high-fat diet tends to become moisture and oily after 2–3 days exposing to air. However, they usually are good for 1 week.
5. Change the diet every week and measure the changes in food weight for measuring the food intake of the mice. Before measurement, pick up the food particle in the beddings.
6. Usually put more food as needed. We do not add fresh food between each measurement as it could result in differences between groups. As experiments continue, high-fat diet groups usually will have more food intake comparing to control groups.
7. When obese mice develop polyuria, it may require changing bedding more than two to three times per week. Make sure that changing bedding will not cause errors in measuring food intake.
8. Perform body weight measurement at the same time while changing bedding (see Note 6)

3.4. Blood Measurement from Tail

1. Insert glucometer strip into glucometer.
2. Pick up mouse with gloves. Restrain the mice with either the other hand or the mice restrainer. We usually use unheated version of rodent restrainer. It s cylindrical and have series of holes on each side to facilitate injections or blood testing. Angle type restrainers are simpler if with experiences.
3. Cut the tail as less as possible with scalpel.

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[5]Regular chow diet from different vendors could have different ingredients. The calories composition is usually protein 20%, fat 10%, and carbohydrates 70%. However, their cholesterol, vitamin, fiber, and fat components might vary. It is important to be consistent between experiments.

[6]Mice are social animals. There are usually some mice that are dominant in the cages and some are not. When measuring body weight, we usually mark which mice are dominant and which are not and compare between groups to get a better insight into their body weight changes.

4. Put one drop of the blood from tail onto glucometer strip and reads.
5. For serum or more blood other than glucose, use capillary tube. If blood stops during capillary tube collection, massage the tail toward the tips.
6. If more blood is needed, collect blood from suborbital area.

3.5. Glucose Tolerance Test

Accurate glucose tolerance test depends on the preparation of the mice. To closely mimic the human part of the glucose tolerance test, we perform mice fasting from 7:00 AM to 15:00 PM. Mice are active during the light–dark phase and this fasting period are more physiological when comparing to human. Without proper fasting, the baseline glucose readings and glucose level after tolerance test will be difficult to interpret.

In our laboratory, we usually administer glucose by intraperitoneal injection. Injection is more consistent with dosage and the results. It also saves time if many mice are going to be tested. However, it is not physiological and cannot totally compare to human glucose tolerance test and omit the effects of glucose on the gastrointestinal tract.

1. Clean the bedding and the food from the cage. Make sure that there is no food left in the beddings. Keep the water bottle.
2. Weigh mice.
3. Prepare a sterile 10% (w/v) glucose solution in PBS. Glucose will be administered at a concentration of 1 g/kg (glucose/ body weight) in a 10 ml/kg volume. Occasionally, we choose 2 g/kg concentration earlier in the experiments before mice become diabetic. 2 g/kg concentration will result in unreadable glucose value in the glucometer if mice become diabetic.
4. Preload 1 ml insulin syringes with the glucose solution. Remove air from the syringes.
5. After fasting period is complete. Measure baseline glucose level. Pick up mouse with gloves. Restrain the mice with either the other hand or the mice restrainer. Cut the tail as less as possible with scalpel. Put one drop of the blood from tail onto glucometer strip and reads. Record glucose value. The range of glucose values is 20–600 mg/dl. If the glucometer indicates the value “Hi”, we usually take the blood with capillary and diluted 2× with PBS. The actual glucose level seldom reaches 1,200 mg/dl and dilution 2× usually is enough to show the exact level.
6. After adequate fasting period, inject mice with intraperitoneal glucose. If too much mice precludes from injection within 15 min, we usually work with two to three people to avoid prolonged injection time.
7. Repeat blood glucose measurements at 15, 30, 60, and 90 min.
8. Return the mice into cages with food.

3.6. Insulin Level Measurement

After serum collection, store the samples at –20°C. Freezing–thawing of the sample, storing the sample at 4°C, or failure to make sure that the kit reaches room temperature will result in experimental error.

1. Prepare reagent. Dilute the conjugate with ten parts of conjugate buffer. Reconstitute insulin control with 0.6 ml distilled water. Dilute 20 ml wash buffer with 400 ml distilled water.

2. Ensure that microplates are at room temperature prior to opening foil pouch. Designate microplate strips for the standards, controls, and mouse samples.
3. Add 10 μl of each standard, control or mouse sample into its respective wells.
4. Add 75 μl of enzyme conjugate into each well. Seal the plate with film provided.
5. Place the microplate in room temperature and shaking at 700–900 rpm for 2 h.
6. Wash the microplate for six times with wash buffer. After the final wash with wash bottle, remove any residual buffer and bubbles from the wells by inverting and firmly tapping the microplate on absorbent paper towels
7. Add 100 μl of substrate into each well.
8. Incubate for 15 min at room temperature on a horizontal microplate shaker.
9. Pipette 100 μl of stop solution into each well. Gently shake the microplate to stop the reaction.
10. Read the absorbance at 450 nm with a reference wavelength of 620–650 nm. Read the plate within 30 min following the addition of the stop solution.

3.7. Glucose Stimulated Insulin Secretion

We usually perform glucose-stimulated insulin secretion test alone with glucose tolerance test. However, glucose stimulated insulin secretion test is more cumbersome and will need at least two people together to perform glucose tolerance test and glucose stimulated insulin secretion test at the same time.

The peak insulin secretion after glucose intraperitoneal injection is around 9–10 min. We typically collected sample at 0, 5, 10, 15, and 30 min after glucose injection. Glucose stimulated insulin secretion test impairment is a key factor in beta-cell failure and some genetic manipulation mice may only show phenotype in this test other than glucose tolerance test.

1. Prepare glucose and measure the body weight of the mice.
2. Collect the baseline blood into a capillary tube.
3. Administer glucose to mice (1 mg/kg in 10 ml/kg volume).
4. Collect the blood at 5, 10, 15, and 30 min after glucose injection.
5. Spin the capillary tubes into a microhematocrit centrifuge at 12,000 g for 10 min. Cut the capillary tubes at the level between serum and red blood cells.
6. Add the serum into the microplates. Samples could be frozen at –20°C for later measurements.
7. Measure the insulin level as previously described.

3.8. Insulin Tolerance Test

The dose of insulin tolerance test may vary from 1 to 5 U/kg and should take into consideration of the body weight, diabetic status and genetic background of the mice. In the C57BL/6J diet induced obesity model, we usually use 2 U/kg insulin for injection. We usually perform insulin tolerance test in fasting condition. However, it sometimes could be performed in the fed condition if target molecules of different models are considered.

1. Perform 7:00 AM to 15:00 PM fasting.
2. Measure the body weight of the mice.

3. Prepare insulin and glucose. Dilute insulin into 2 U/10 ml by iced sterile PBS. Preload the injection syringes with insulin and keep them on ice. Prepare one or two glucose sterile solution to prevent hypoglycemia of the mice.
4. Collect the blood at 0, 15, 30, 60, 90, and 120 min after insulin injection.
5. Measure glucose level and observe the mice. If the mice are ill-looking, inject glucose immediately to avoid mortality.

3.9. Body Composition Measurement

With the advances of the technology, magnetic resonance imaging (MRI) and dual energy X-ray absorptiometry scanning (DEXA) has gradually replaced the chemical carcass analysis for measuring the fat component (21). Chemical carcass used to be considered as the gold standard for whole body composition analysis. It requires to sacrifice the mice and time-consuming. Dissecting and weighing of fat tissues in individual mice can also measure the total body fat. However, this method is less accurate. The collection of visceral fat can be challenging, as it is often integrated in the internal organs and cannot be accurately dissected.

Body composition measurements by DEXA consist of three components: fat, bone mineral, and lean soft tissue. It shows strong correlation between the percentage of fat area and total body fat as compared with chemical extraction method. There is no need for sacrifice the mice and can be used for serial measurement.

1. Measure the animal's body weight and anesthetized the mice with ketamine/xylazine solution, intraperitoneally.
2. Calibration for the bone mineral density and percent fat content using the phantom mouse unit.
3. Place the mice ventral side down on disposable plastic trays so that the whole mouse can be measured.
4. Record bone mineral density, bone mineral content, lean mass, fat mass, and percentage body fat.
5. Analyze the data using PIXImus2 software.

3.10. Rapamycin Treatment of Mice

1. Dissolve 1 mg Rapamycin in 20 μl ethanol for stack solution.
2. Further dilute dissolved Rapamycin with Ringer's saline solution to a final concentration of 1 mg/ml directly immediately before use.
3. Restrain the mouse and expose the abdomen.
4. Prepare the 25 gauge needle and fill the syringe with diluted Rapamycin.
5. Disinfect the injection site and insert the needle cranially at 30° angle caudal to the umbilicus and lateral to the midline.
6. Before each injection, make sure to aspirate before injection to avoid penetration to intestine or gall bladder.

3.11. Mice Sacrifice and Collection of Tissue and Blood

After the diet feeding experiments, mice are sacrifice to collect their blood, internal organ and analyze the whole body composition. Tissue also will be used for further analyze, including protein or RNA or histology section.

Diet induced obesity model will result in multiple organ changes when comparing to its control mice. High-fat diet will lead to hepatic steatosis and fatty liver changes could be obviously observed after dissection. Adipocyte number and size are increased. Pancreatic islets will become hypertrophic and then degenerate. Skeletal muscle may have decreased insulin sensitivity. Brown fat may show atrophy or white fat deposition. Hence, obesity is a whole body system derangement. Although some organ systems are affected more than other systems, we usually collect involved organs in sequence to avoid loss of data.

1. Prepare the sacrifice plates, syringes, eppendorf tube, dissection tools, perfusion tools, and iced PBS solution before sacrifice.
2. Mark every eppendorf tubes for serum ample, tissue collection or histological fixation. Fill the 3.7% formaldehyde or tissue lysis buffer or RNA extraction solution (Trizol) before sacrifice.
3. Euthanize the mice by CO_2 according to the animal protocol and ethical regulation.
4. Fix the extremity of the mice with needle to dissection plate.
5. Make an incision through the skin horizontally near the umbilical area.
6. Remove the skin and subcutaneous layer.
7. Make a vertical incision through the midline abdomen to open the abdomen.
8. Cut through the chest cavity and reveal the heart and lung.
9. Insert a needle at the left ventricle apex of the heart and draw the blood slowly. Avoid drawing the blood too soon. 300–1,000 μl blood can be obtained in one mouse.
10. Collect the blood into blood collection eppendorf tubes.
11. Collect pancreas first, as their RNA and protein are more prone to damage by pancreatic enzyme.
12. Collect heart, lung, liver, spleen, kidney, and intestine if needed.
13. Collect subcutaneous, inguinal, and mesenteric white fat pad.
14. Collect brown fat at the back.
15. Collect the brain if indicated.
16. Proceed with histology or tissue analysis (see Note 7).

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[7]For comparing white adipose tissue with histological section, it requires to comparing multiple location of the white fat with serial section for more than 20 slides to get accurate data. We usually collect subcutaneous, inguinal, mesenteric, gonadal, and white fat nearby brown fat to get overall view for white fat differences between mice.

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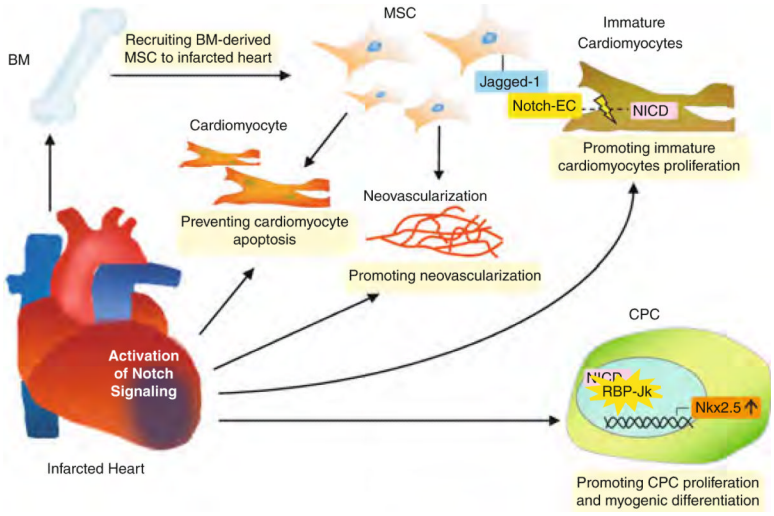

Fig. 1.

(**a**) Schematic diagram showing how we design the high-fat and chow diets feeding with and without treatment. In this experiment, we use rapamycin to test if body weight is affected by 4 weeks treatment of rapamycin. (**b**) The typical body weight chart when mice fed with high-fat diet or chow diet (Adapted from Wang et al. Science Signaling (2009) with permission).